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CEREBROSPINAL FEVER

Studies in the Bacteriology, Preventive Control, and Specific Treatment of Cerebrospinal Fever among the Military Forces, 1915-19

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CEREBROSPINAL FEVER

Studies in the Bacteriology, Preventive Control, and Specific Treatment of Cerebrospinal Fever among the Military Forces, 1915–19



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INTRODUCTION

In the present Report Dr. M. H. Gordon has brought together, at the request of the Medical Research Committee, the results of the scientific work done by himself and his colleagues from early in 1915 up to the summer of 1919 towards the investigation of cerebrospinal fever among the military forces, and the application of the knowledge so gained to the preventive control and the curative treatment of the disease. This account includes the observations already recorded in Report No. 3 of the present series, which gave the results of the work done during the first two years, 1915 and 1916, of this inquiry. That earlier Report is now out of print: on that account and for other reasons it seemed best that those preliminary results should now be resumed here in the light of the experience of practical measures successfully based upon them, and of the further inquiries to which they led. Included here also, and now for the first time published, are two Reports—that by Captain Glover upon 'Military Overcrowding and the Meningococcus Carrier Rate' (p. 133), and that by Major Hine upon the 'Specific Treatment of Cerebrospinal Fever by Monotypical Sera' (p. 176), which were printed and issued by the Committee in 1918 and 1919 respectively, for the information of our own and the Allied naval and military forces and of the civilian health services.

These two papers may be said to represent the two main directions of practical application, one preventive, the other curative, for which the earlier studies by Lieut.-Colonel Gordon of the natural history of the infective meningococcus had been the indispensable preliminary. Fuller accounts are now given of these two main branches of work. Captain Glover's studies of the 'carrier-rate' in a community as an index of overcrowding and of a rise in 'carrier-rate' as a danger-signal preceding an outbreak of disease, not only have great interest as a contribution to epidemiology, but have given practical methods of control of which the success has been put beyond doubt. His interesting conclusions on p. 163 show, as Dr. Gordon remarks, that 'the best way to prevent cerebrospinal fever is to prevent overcrowding'. This is a good example of the manner in which accurate and laborious scientific work may lead to a practical conclusion which appears to be self-evident and at which there is a common temptation to think that 'common sense' might have arrived, unaided by any special investigation. Yet, for practical military purposes 'overcrowding' is only a relative term, meaningless by itself. As the result of the work here described we can now define with striking and certainly unexpected accuracy the point at which crowding becomes overcrowding in respect at least of this disease. 'Common sense' in matters of this kind too often has only a value like that of prophecy after the event; it is a poor guide to practice until it has the stimulus and support of accurate knowledge.

Upon the curative side Lieut.-Colonel Gordon's work was in the more recent period aimed at using the knowledge he had gained of the different 'types' of the meningococci to producing an effective anti-endotoxic serum for each type, so that the most effective serum could be applied in treatment of the disease, and with the maximum potency for the particular type discovered in the individual patient. The production of the serum was carried out on behalf of the Committee by Dr. Stanley Griffith at the Field Laboratories, Cambridge, who worked throughout in close consultation with Lieut.-Colonel Gordon at Millbank. It will be seen that Dr. Griffith was successful in producing a highly potent serum against Type I endotoxin, but it has so far proved to be much more difficult to produce a satisfactory anti-endotoxin for Type II. The remarkable results in life-saving already gained by the use of this curative serum, and especially when it has been used accurately in respect of a diagnosed type and used early in the disease, are given on page 182. Since the closing of Dr. Gordon's work at Millbank last year, the Medical Research Committee have arranged for the continuation of this serum production at Cambridge by

Mr. E. G. D. Murray, who is working at the more refractory Type II under Dr. Gordon's general direction.

It is generally impossible to measure accurately the value of preventive work; too commonly, the more successful it is the less is heard and thought of it. There can be no serious doubt, however, that great saving of life and the avoidance of impending and possibly disastrous epidemics of cerebrospinal fever were effected among the Home Forces during times and conditions of special danger by the administrative measures carried out by the Army Medical Service, and guided by the knowledge gained by the scientific men whose services the Committee were privileged to contribute. The Committee would here again desire to express their cordial acknowledgements of the effective arrangements made by the Army Medical Service for bringing the resources of civilian workers into the best relation to administrative and military problems. The continuous give and take between the workers in the laboratory and those in the practical field, and the continual exchange of stimulus between them, provided a model of successful effort that should have many fruitful analogies in the future.

The actual curative work effected in the Armies, unlike the preventive work, is capable of some direct measurement. A patient in civilian life has much less than half a chance of escaping death when attacked by cerebrospinal or 'spotted' fever. The case mortalities among civilians of all ages for the five years 1914 to 1918 inclusive have been close to 69 per cent., 65 per cent., 65½ per cent., 65½ per cent., 67½ per cent. respectively. For the purposes of comparison with the mortality in troops, we may take as the most favourable the mortalities of those civilians between the ages of 20 and 30 only; for the last four of the years mentioned these have been close to 63, 51, 64½, and 58 per cent. respectively. The treatment of men of the Home Forces during the same five years gave, however, the following case mortalities in succession respectively, 65 per cent., 49 per cent., 44½ per cent., 44⅓ per cent., and 35½ per cent. In 1914, that is to say, a man attacked by the disease had on the average only one chance in three of escaping death, whether he was a civilian or a soldier. But in 1918, while the civilian had the same high risk, a soldier had now two chances in three of living: his chance of life was doubled. The progressive improvement shown by the military results was due no doubt to early diagnosis and treatment, the proper use of lumbar puncture, and, especially in the last year, to the use of an effective serum. In 1918–19 an attacked soldier, if his disease was of Type I and serum was available, was given close to nine chances out of ten of escaping death. It is to be hoped that the civilian health services and the medical profession at large will soon bring to the aid of the civilian population in general the methods of early diagnosis and specific treatment which the Army Medical Service had so successfully made available for the Forces before the close of the war.

The Committee would offer their warmest acknowledgements for the preparation of this Report to Dr. Gordon (late Hon. Lieut.-Colonel, R.A.M.C.), who, with Dr. T. G. M. Hine (late Hon. Major, R.A.M.C.), has been working at this subject on their behalf from the beginning in 1915; and no less to his other colleagues, Majors A. S. G. Bell and Martin W. Flack, Dr. Stanley Griffith, Captains J. A. Glover and E. G. D. Murray, and Major W. J. Tulloch, who, whether in the Army Medical Service or working for the Committee, have taken various important parts in the work, and some of whom have contributed as shown in the text to the present account of it.

Medical Research Committee,
15, Buckingham Street,
Strand, W.C. 2.

March 29, 1920.

CEREBROSPINAL FEVER

STUDIES IN THE BACTERIOLOGY, PREVENTIVE CONTROL, AND SPECIFIC TREATMENT OF CEREBROSPINAL FEVER AMONG THE MILITARY FORCES, 1915–19

CONTENTS

PART I. MEASURES ADOPTED WHEN THE OUTBREAK BEGAN

PAGE

1. Introduction 9
2. State of knowledge at the beginning of the outbreak with regard to the Bacteriology of Epidemic Meningitis or Cerebrospinal Fever 11
   - A. Relationship of the Meningococcus to the Disease 11
   - B. Diversity among Meningococci 11
   - C. The mode of spread of Cerebrospinal Fever. Carrier Rates . . 12
   - D. Means of identifying the Meningococcus 12
   - E. Pathogenesis 13
   - F. Antimeningococcus Serum 13
3. Measures adopted for limiting the spread of Cerebrospinal Fever in the Army when the outbreak began in 1915 . . 13

PART II. STUDIES OF THE MENINGOCOCCUS

4. Search for a suitable Medium for Culture of the Meningococcus 15
5. Serological Definition of the Meningococcus 17
6. The Pathogenicity of the Meningococcus 20
   - A. The Effect of Various Measures on the Pathogenicity of the Meningococcus 22
     - (*a*) *Pathogenicity of the same Meningococcus when alive, and when killed by Ether and by Heat respectively* 22
     - (*b*) *The Effect on its Pathogenicity of suspending the living Meningococcus in broth and in distilled water respectively* . . . 22
     - (*c*) *The Effect on its Pathogenicity of killing the Meningococcus by Desiccation* 23
     - (*d*) *The Effect of Age on the Pathogenicity of the Meningococcus* . 24
     - (*e*) *Conclusion* 24
   - B. Search for evidence of the Secretion of a Soluble Toxin by the Meningococcus 25
     - (*a*) *Search for Extracellular Toxin in Cultures in vitro* . . . 25
     - (*b*) *Search for Extracellular Toxin in Cultures in vivo* . . . 25
7. Intracellular Constituents of the Meningococcus of pathogenic importance 27
   - A. Studies of an Intracellular Haemolytic Substance contained by the Meningococcus 27
     - (*a*) *Haemolytic Action of the Meningococcus when growing in Culture* 28
     - (*b*) *Influence of Age on the Haemolytic Power of the Meningococcus* 30

PAGE

(c) *Diffusibility of the Haemolytic Substance* 30
(d) *Effect of Autolysis in setting free the Haemolytic Substance* . 30
(e) *Effect of Heat on the Haemolytic Substance* 31
(f) *Effect of Desiccation on the Haemolytic Substance* . . . 31
(g) *Effect of Solvents on the Haemolytic Substance* . . . 32
B. Studies of an Intracellular Reducing Agent contained by the Meningococcus 33
(a) *Comparison of Red Blood Corpuscles and Methylene Blue as Indicators of the Reducing Action of the Meningococcus* . . 34
(b) *Effect of Menstruum on the Reducing Power of the Meningococcus* 35
(c) *Is the Reducing Agent soluble in the Menstruum or confined to the body of the Meningococcus?* 35
(d) *Effect of Heat on the Reducing Power of the Meningococcus* . 36
(e) *Effect of Age on the Reducing Power of the Meningococcus* . . 38
(f) *Effect of Desiccation on the Reducing Power of the Meningococcus* 38
(g) *Effect of Menstruum on the Vitality of the Meningococcus* . 38
(h) *Effect of Antiseptics on the Vitality and Reducing Power of the Meningococcus respectively* 39

8. Further Observations on the Reductase and Endotoxin of the Meningococcus 41
A. Comparison of the Reductase of the Meningococcus with that of *Staphylococcus epidermidis* and of *B. Coli* respectively . . . 41
(a) *Effect of Exposure to a Temperature of* 70° *C.* 41
(b) *Effect of Desiccation* 42
B. Serviceability of the Reductase for detecting the presence of living Meningococci in Body Exudates 42
C. The Endotoxin of the Meningococcus 43
(a) *Experiments to determine* (1) *if the virulence of the Meningococcus can be increased by animal passage; and* (2) *if in the event of such increase of virulence, the Endotoxin contained by the Coccus is also increased* 45
(b) *Comparison of the value of various Solvents for extracting the Toxin from the dried Meningococcus* 45
(c) *The Value of improved Mechanical Disintegration for extracting the Endotoxin from the dried Meningococcus* 46
(d) *The Value of Sodium Hydroxide for getting the Endotoxin of the Meningococcus into Solution* 47
(e) *The Value of Autolysis for the purpose of obtaining a Solution of Meningococcus Endotoxin* 48
(f) *The Effect of Heat on the Endotoxin of the Meningococcus* . 48
(g) *Trial of Vaughan's Method for the purpose of obtaining a Solution of the Endotoxin of the Meningococcus* 51
(h) *A Method of obtaining the Endotoxin in Solution* . . . 52

9. Antibodies to the Meningococcus 53

10. Agglutinin 53
A. Antigens 53
B. Mode of Dosage 54
C. Mode of conducting the Agglutination Test 54
D. Absorption Tests 55
E. Agglutinogenic Values of various Preparations of the same Meningococcus 55
F. The Effect of Autoclaving the Meningococcus on its Antigenic Specificity 56

PAGE

11. Some Observations on Meningococcal Agglutinating Sera and their Production. By Major A. S. G. Bell, R.A.M.C. . . . 57

12. On a Rapid Method of determining the Serological Type of a Meningococcus by the Agglutination Test. By Major A. S. G. Bell, R.A.M.C. 63

13. Complement-fixing Antibodies to the Meningococcus. By Major A. S. G. Bell, R.A.M.C. 66

14. The Capacity of Antimeningococcic Serum for inducing Phagocytosis. By W. J. Tulloch, O.B.E., M.D., Brevet-Major R.A.M.C. . 76
A. Technique 77
B. Estimation of the Activity of Serum 78
C. Discussion 81

15. Lysin 81
A. The Literature concerning Lysin to the Meningococcus . . . 81
B. Object of the Present Investigation 83
C. Studies with a View to obtaining a Method of estimating Lysin *in vitro* 84
D. Investigation of the Lysin Content of Samples of Antimeningococcus Serum 90

16. Antiendotoxin 93
A. Manner in which the importance of Antiendotoxin came to notice during the present outbreak 93
B. The Present state of Knowledge concerning Meningococcus Antiendotoxin 96
C. Mode of testing Serum for Meningococcus Antiendotoxin . . . 98
D. Experimental Observations on the production of Meningococcus Antiendotoxin 101
E. The Valency of Meningococcus Antiendotoxin 105
F. Clinical Results with the Monotypical Sera 108
G. Conclusions with regard to Antiendotoxin 108

17. The Protective Power of Antimeningococcus Serum . . . 109

18. A study of Gram-negative Diplococci of the Nasopharynx resembling the Meningococcus. By W. J. Tulloch, O.B.E., M.D., Brevet-Major R.A.M.C. 111
A. Introduction 111
B. Material and its Source 112
C. Cultural Examination of the Nasopharyngeal Micro-organisms . 113
D. Investigation of Representative Specimens of the Series by cultivating them in Carbohydrate Media 114
E. Examination of the 40 Gram-negative Cocci of Pharyngeal origin by the Agglutination and Absorption of Agglutinins Test . . . 115
F. Are the Agglutination Reactions of these Pharyngococci Constant ? 119
G. Conclusions which may Tentatively be drawn from the Results obtained on Investigating the Series of Micro-organisms under Consideration by the standard Agglutination and Absorption of Agglutinin Technique 121
H. Investigation of Representative Pharyngococci and Representative Type Cultures of Meningococci, using Sera of Animals—Rabbits—immunized against Pharyngococci 122
I. Agglutination of Representatives of the present Series of Cultures with Polyvalent Antimeningococcus Horse-serum, prepared for Therapeutic Purposes 126
J. Conclusions 127

PART III. APPLICATION OF THE PRECEDING AND FURTHER RESEARCHES TO THE PREVENTION, DIAGNOSIS, AND SPECIFIC TREATMENT OF CEREBROSPINAL FEVER

PAGE

19. The Prevalence and Distribution of Individual Types of the Meningococcus 128
A. Meningococci isolated from the Cerebrospinal Fluid of Cases of Cerebrospinal Fever 128
(*a*) *Proportion of Meningococci identified by the Agglutination Test* 128
(*b*) *The Frequency of each Type* 128
(*c*) *Sources of these Meningococci* 129
(*d*) *Result of the Absorption Test* 130
(*e*) *Result of the Examination of a Plurality of Colonies in Primary Cultures from the Cerebrospinal Fluid of Cases of C.S. Fever* . 130
B. Meningococci isolated from Nasopharyngeal Secretion 130
(*a*) *The Nasopharyngeal Secretion of Cases* 130
(*b*) *The Nasopharyngeal Secretion of those in the Environment of the Case* 132

20. Observations of the Meningococcus Carrier Rate, and their Application to the Prevention of Cerebrospinal Fever. By J. A. Glover, O.B.E., M.D., D.P.H. 133
A. Definition of Terms used 133
B. Introduction 134
C. The Close Relation between Overcrowding and Carrier Rate . . 134
D. Military Overcrowding in the War Emergency 135
E. Overcrowding in Sleeping Quarters a factor of Paramount Importance in the Production of High Carrier Rates 136
F. Space between Beds and Carrier Rate 138
G. 'The Warning Rise' in the Non-contact Carrier Rate . . . 140
H. Correspondence of Prevalent Carrier Type with Prevalent Case Type 153
I. Higher Carrier Rate and Mass Infection 155
J. The Rapid Reduction of Carrier Rate by increasing the distance between Beds 156
K. Carrier Rates in Aylwin Huts 157
L. Tents 159
M. Emergency Prophylaxis consequent upon Determination of Carrier Rate 160
N. Corners and Partitions 162
O. Conclusions 163

21. Mass-Disinfection of the Nasopharynx 165
A. Preliminary Experiments 167
B. Observations on the Value of Inhaling-Room Treatment for clearing up Meningococcus Carriers 169
(*a*) *Results with Chloramine T.* 169
(*b*) *Results with Zinc Sulphate* 169
C. The Value of Inhaling-Room Treatment for Mass-Disinfection of the Nasopharynx 170

22. Improved Atomizer for Mass-Disinfection. By T. G. M. Hine, O.B.E., M.D., Major R.A.M.C. 173

23. An Analysis of the Result of the Therapeutic Application of Monotypical Antimeningococcus Serum in 267 cases of Cerebrospinal Fever. By T. G. M. Hine, O.B.E., M.D., Major R.A.M.C. . 176

24. The Significance of the Serological Types of the Meningococcus 188

25. Bearing of the Preceding Observations on the Prevention, Diagnosis, and Specific Treatment of Cerebrospinal Fever . . 199

26. References and Recent Bibliography 201

PART I. MEASURES ADOPTED WHEN THE OUTBREAK BEGAN

1. Introduction.

The researches described in the present report were carried out in the Central Military Laboratory for Cerebrospinal Fever during the years 1915–19 while that disease was manifesting itself with varying frequency amongst troops in training in this country in course of the Great War. The investigations made during the first two of these years have been described in Report No. 3 of this series ; but as this publication is now out of print, the ground covered by it is included in the present report.

The studies of the meningococcus related in the following pages were undertaken with a view to applying their result for the purpose of increasing the efficacy of the bacteriological measures adopted for dealing with cerebrospinal fever in the army. To this end, shortly after the bacteriological organization was in working order, and a suitable medium had been arrived at for cultivation of the meningococcus, a systematic serological survey was made of the meningococci concerned in the outbreak in order to improve the accuracy of the bacteriological procedure in operation for limiting the spread of the disease by the identification of carriers, and in order also to define the basis of a correct system of serotherapy. The accuracy of the differentiation then effected by means of the agglutination test checked by the absorption test was confirmed by the continuous experience of the following three and a half years, during which the intermittent occurrence of cerebrospinal fever continued to provide specimens of the meningococcus for examination. Later, in view of the severe mortality amongst those contracting the disease, and in view especially of the lack of success of much of the antimeningococcus serum then available, a special effort was made to obtain serum of greater potency. No sure method of standardizing antimeningococcus serum being known, a study was undertaken of the pathogenic properties of the meningococcus, and of the means of defence against it, with special reference to individual antibodies concerned in the therapeutic potency of antimeningococcus serum. By comparing the antibody content of a sample of serum that had proved clinically to be remarkably successful with that of other specimens of serum of far inferior clinical value, it was found that one antibody in particular—antiendotoxin—is associated with therapeutic potency, and confirmatory evidence of this association was soon forthcoming. Information having been obtained in this way as to the quality of serum required for clinical efficacy, a special study was made of the conditions under which antiendotoxin is formed, and an attempt was made to prepare therapeutic serum containing it, with what measure of success will be seen in the report.

During these investigations some new points came to light with regard to the physiology of the meningococcus. The interest attaching to the haemolytic substance contained by this micro-organism,

and to its intracellular reductase (both of which are now described for the first time) is hardly less from a general point of view, than that excited by the problem of the antigenic specificity of the different types of the meningococcus, and of their respective proteins and endotoxins, because of its bearing on the control and successful treatment of cerebrospinal fever.

This introduction would be incomplete did it fail to state that throughout the four years occupied by these investigations every possible help was given by the Medical Research Committee and by the War Office. Without the co-operation and researches of Major T. G. M. Hine, O.B.E., and Brevet-Major W. J. Tulloch, O.B.E., of Captain E. G. Murray, O.B.E., in the early stages, and of Major A. S. G. Bell in the later, but little progress would have been possible. The situation demanded that any advance in knowledge that could be effected should be applied at once for dealing with the disease. Accordingly, as soon as a suitable medium had been arrived at for culture of the meningococcus, and the meningococci at work in the outbreak had been defined by the agglutination test, the Supply Department of the Central C.S.F. Laboratory took over the manufacture and supply of trypagar, enrichment material, agglutinating serum, and standard suspensions to district laboratories ; and later this department also undertook the distribution of the special therapeutic serum, and an analysis of the clinical results. The manner in which the Supply Department met the severe demands made upon it is entirely due to the organizing capacity, ability, and foresight of Major Hine who was in charge of it throughout. Acknowledgment is also due to Lieut.-Colonel Martin Flack, C.B.E., R.A.F., for his help with regard to the medium, and for his initiation of those continuous studies of the carrier rate which his successor in the London District C.S.F. Laboratory, Captain J. A. Glover, O.B.E., has continued, improved upon, and applied with such striking success to the prevention of cerebrospinal fever. Special thanks are also due to Dr. Stanley Griffith for his strenuous and persevering effort to succeed in the very difficult task of preparing therapeutic serum of the quality desired. Lastly, acknowledgement must be made of the valuable services of Corporal J. Heagerty and Sergeant C. S. Barrett, to whom fell latterly so many of the routine laboratory observations.

The researches and observations described in this report have been arranged under three chief headings. In addition to the present Introduction, Part I contains a brief description of the state of knowledge on the subject of the bacteriology of cerebrospinal fever at the beginning of the outbreak, and also of the measures taken to deal with this disease when it broke out among the numerous recruits undergoing military training during the early months of 1915. Part II contains a series of investigations of the meningococcus and of the means of defence against it—studies undertaken with a view to dealing more effectively with the disease to which this micro-organism gives rise. In Part III the application is considered of these and further researches to the prevention, diagnosis, and specific treatment of cerebrospinal fever. Articles by Majors Bell, Hine, and Tulloch, and by Captain Glover, on subjects to which they have devoted special attention and research are included in the report.

2. State of knowledge at the beginning of the outbreak with regard to the Bacteriology of Epidemic Meningitis or Cerebrospinal Fever.

Before proceeding to describe individual researches carried out in the Central Laboratory during the recent outbreak, it is desirable to refer briefly to the position of bacteriological knowledge on the subject of cerebrospinal fever at the point when the outbreak started in the early months of 1915.

A. *Relationship of the Meningococcus to the Disease.*

Since Weichselbaum discovered the meningococcus in the meningeal exudate of six cases of cerebrospinal fever in 1887, the presence of this micro-organism had been verified in every outbreak of cerebrospinal fever where adequate bacteriological investigations had been made, as also had its absence from the tissues of patients suffering from other diseases. In addition to this constant association of the meningococcus with cerebrospinal fever, the experimental observations of Flexner, v. Lingelsheim and Leuchs, Stuart M'Donald and others on monkeys had shown that when introduced into the cerebrospinal system, the meningococcus is capable of producing the most characteristic lesion of cerebrospinal fever, namely meningitis. For these reasons the meningococcus had come to be accepted as the essential infective agent of cerebrospinal fever.

B. *Diversity among Meningococci.*

At first meningococci were regarded as comprising a single and undivided group. Among the earliest to observe serological differences between individual meningococci were Kutscher, Arkwright, and Elser and Huntoon. The first observer, however, to attempt to definitely classify meningococci by their serological characters was Dopter who, in 1909, found in the nasopharyngeal secretion of contacts a group of Gram-negative cocci that, though indistinguishable from the meningococcus in morphological, cultural, and fermentative characters, and also in the fixation of complement test, were nevertheless distinguished from it by the fact that they were not agglutinated by antimeningococcus serum, and also by the precipitin test when the results were checked by the absorption method. Dopter concluded that these pharyngococci were distinct from the meningococcus, and suggested the name parameningococcus for them. On proceeding to examine on similar lines meningococci isolated from patients suffering from cerebrospinal fever, Dopter found that some of them were examples of the parameningococcus, and in 1911 he published a paper in which he stated that he had collected seven cases of sporadic meningitis all of which ended fatally, were unaffected by antimeningococcus serum, and were due to the parameningococcus. During 1912 Dopter came to the conclusion that there was more than one kind of parameningococcus, and in 1914 he, by means of the agglutination test, checked by the absorption test, succeeded in differentiating seven specimens of the parameningococcus into three groups as follows : parameningococcus, (*a*) three specimens, (*b*) three specimens, and (*c*) one specimen. These researches of Dopter, therefore, had shown that in spite of a general similarity with regard to

their morphological, cultural, and fermentative characters, considerable diversity exists among meningococci in respect of their serological characters. In addition to the meningococcus, he, by application of the agglutination test. checked by the absorption test, had succeeded in differentiating three different kinds of parameningococcus. As yet, however, the relative frequency of these serological groups of the meningococcus and their relation to epidemic meningitis were unknown.

C. *The mode of spread of Cerebrospinal Fever. Carrier Rates.*

Owing to the fact that during an outbreak of cerebrospinal fever as a rule no direct relation can be traced between individual cases, the mode of spread of this disease was for some time obscure. The mystery, however, was cleared up by the discovery of the meningococcus in the nasopharyngeal secretion not only of cases, but also of contacts ; thus explaining the mode of spread of cerebrospinal fever through a chain of healthy carriers until a susceptible person is reached. An excellent summary of the information available on this matter at the beginning of the outbreak is contained in the chapter on ' the nasopharynx as the habitat of the meningococcus ' in the well-known book of Netter and Debrè (*La Méningite Cérébrospinale*. Paris, 1911). The cultivation of the meningococcus from the nasopharyngeal secretion had been found to be liable to many sources of error ; but when these were excluded it appeared that this microorganism is constantly to be found in the nasopharyngeal secretion of the patient during the first days of the malady. The carrier rate amongst those in the vicinity of the patients had been found to vary ; some observers reporting 5 per cent. of carriers among the contacts, others as many as 50 or 60 per cent. The explanation of these different rates appeared to lie in the technique, the stage of the outbreak when the contacts were examined, season, and hygienic conditions—overcrowding having a marked influence in increasing the carrier rate. From the information available, the conclusion arrived at by Netter and Debrè was that the intensity of the outbreak and the carrier rate correspond. It was pointed out also that control observations in regions free of cerebrospinal fever had shown a relative scarcity of the meningococcus, the normal carrier rate being about 2 per cent.

D. *Means of identifying the Meningococcus.*

In cerebrospinal fluid the meningococcus is relatively easy to detect, because when found there it is usually in pure culture. In nasopharyngeal secretion, on the other hand, the meningococcus was known to be far more difficult to identify because of the presence there of a number of Gram-negative cocci with which it is liable to be confounded. The morphological, cultural, and fermentative characters of the meningococcus had been found to be of value for differentiating it from many of these pharyngococci ; but their limit for this purpose had not yet been adequately defined. According to v. Lingelsheim and Kutscher the agglutination test was of most value for identifying the meningococcus in nasopharyngeal secretion. The macroscopic method of conducting the test was recommended, and it had been found advisable to incubate the tubes at 55° C. rather than at 37° C

Raymond Koch also had drawn attention to a point of much practical value, namely, that suspensions of meningococci in saline, if heated for 30 minutes to 65° C. and then phenolated, retain their antigenic and agglutinating capacity for weeks and even months, so that it is unnecessary to use a fresh culture for each test. Kutscher, and Elser and Huntoon also had drawn attention to the advantage of using young rabbits when preparing agglutinating serum against the meningococcus.

E. *Pathogenesis.*

Albrecht and Ghon, Jundell, Rist, and others had all searched for the presence of soluble toxin in cultures of the meningococcus without success. The pathogenic action of the meningococcus was regarded by Flexner and others as due to its endotoxin, which he, Kolle and Wassermann, Dopter, and many others had found to be liberated by subjecting the coccus to autolysis. A further factor was the capacity of the coccus to multiply in the tissues of its host. As a result of his valuable experimental studies of the pathogenicity of the meningococcus, Flexner had formed the opinion that the meningococcus ' acts in causing death probably through disintegration and not to a secreted extracellular poison '. He had also observed that ' multiplication on the part of the cocci is not at all essential to the production of the peculiar symptoms and lesions. Even though multiplication of the virulent diplococci does take place, it is still likely that the toxic action is caused by the cocci which have suffered dissolution.' It may be noted also that Flexner found that heating the autolysate to 65° C. does not materially reduce its toxicity.

F. *Antimeningococcus Serum.*

Before the beginning of the recent outbreak it had been demonstrated conclusively by Flexner, Dopter, and others that suitable antimeningococcus serum when administered intrathecally in adequate quantity, with sufficient frequency, and as soon after the onset as possible, is capable of reducing the mortality from cerebrospinal fever in a very striking manner. Antimeningococcus serum had been found to contain complement-fixing antibodies, agglutinin, precipitin, opsonin, lysin, and antiendotoxin. No general agreement, however, had yet been reached with regard to the best method of standardizing the serum, Kolle and Wassermann recommending standardization by the complement-fixing antibodies, Neufeld and Jobling by the opsonin content, and Kraus and Doerr by the antiendotoxin content. It may be added that since the recent outbreak began Nicolle, of the Pasteur Institute, has declared himself in favour of the standardization of antimeningococcus serum by complement fixation, while Amoss of the Rockefeller Institute prefers to use agglutinin for this purpose.

3. Measures adopted for limiting the spread of Cerebrospinal Fever in the Army when the outbreak began in 1915.

When cerebrospinal fever broke out among troops in training in the early months of 1915, and spread rapidly, special measures became necessary for limiting its spread in the army. These measures were under the direction of Brigadier-General Sir William Horrocks, K.C.M.G., C.B., assisted by Colonel R. J. Reece, C.B. The procedure adopted was as follows : upon the occurrence of a case in

which the symptoms were suggestive of cerebrospinal fever the district bacteriologist was called in, and if the diagnosis was confirmed bacteriologically the patient was isolated and the contacts segregated and swabbed. A contact consisted of any one who had been within 3 yards of the case under conditions liable to acquire infection. Those contacts whose nasopharynx failed to yield a micro-organism resembling the meningococcus were returned to duty with the smallest possible delay, while those who were found to be carrying a micro-organism indistinguishable from the meningococcus were kept in isolation until their nasopharynx proved negative on two successive examinations separated by an interval of several days.

Apart from the isolation of the case therefore, the chief feature of this preventive procedure was the search for the meningococcus in the nasopharynx of contacts. For the purpose of obtaining nasopharyngeal secretion, the covered swab introduced by C. E. West was used. In differentiating the meningococcus from pharyngococci, the points upon which stress was laid were (1) the characteristic appearance of its colonies, and (2) the fact that unlike the majority of pharyngococci, the meningococcus as a rule fails to grow on nasgar at a temperature below 25° C. Since the meningococcus ferments glucose with an acid reaction and fails to ferment saccharose, while the majority of the pharyngococci either ferment both sugars, or ferment neither, investigation of the fermentative characters was recommended for confirmatory purposes in the case of contacts whose nasopharynx yielded a micro-organism resembling the meningococcus.

The machinery set up for carrying out this preventive procedure consisted of a central laboratory in London and some fifty district laboratories co-opted or instituted for the purpose throughout the commands. In this manner a special bacteriological service was brought into operation for dealing with any outbreaks of cerebrospinal fever that might occur among troops in training in military camps throughout the United Kingdom.

The immediate functions of the Central Laboratory on its inception were to supply district laboratories with swabs and with special medium for cultivation of the meningococcus. A further duty was the instruction of officers about to take charge of district laboratories in the procedure for identification of the meningococcus in the nasopharynx. This was necessary because at that time none of these bacteriologists had any previous experience of this work.

It was realized from the outset, however, that the bacteriological procedure adopted in the emergency for identifying the meningococcus in nasopharyngeal secretion merely represented the best that could be done under the circumstances, and in the state of knowledge then obtaining ; and that the success or failure of this attempt to apply bacteriology for the purpose of limiting the spread of cerebrospinal fever among troops, and of dealing with the disease generally in the army, would depend mainly upon the result of research. In reality, therefore, one of the chief functions of the Central C.S.F. Laboratory, from the beginning and throughout the whole of the four years of its existence, has been the prosecution of research with a view to the application of information thus obtained for the purpose of improving the procedure in use for dealing with cerebrospinal fever. These researches will now be described.

PART II. STUDIES OF THE MENINGOCOCCUS

4. Search for a suitable Medium for Culture of the Meningococcus.

It was clear that but little progress could be made until a satisfactory medium had been arrived at for culture of the meningococcus. As a medium consisting of a mixture of nutrose, serum, and agar (nasgar) proved successful in some preliminary observations, and had the advantage that it could be prepared without difficulty on a large scale with the comparatively inexperienced labour then available, this medium was made up and sent out in the first place to district laboratories. Meantime, an investigation was carried out at the Central Laboratory for the purpose of defining the conditions of growth *in vitro* of the meningococcus with a view to the elaboration, if possible, of a better medium for routine purposes.

The chief requirements that a satisfactory routine solid medium for the meningococcus should meet were formulated as follows :

(1) It must grow the meningococcus with constancy in primary culture as well as in subculture.
(2) It must be transparent, so that colonies of the meningococcus developing upon it can readily be distinguished from those of other cocci.
(3) It must be simple and easy to make, sterilize, and handle, and as far as possible free of materials of foreign origin.
(4) When the supply of a large number of laboratories is in question, as in the present instance, it must be cheap.
(5) If possible, the medium should be capable of maintaining the meningococcus alive for a considerable time ; thus obviating the necessity of frequent subculture.

As details of this investigation have been published elsewhere, only its main features and results will here be considered. Starting from the central fact that ordinary agar culture medium does not become a satisfactory medium for the meningococcus until it has received an enrichment of animal protein in some form or other, a menstruum was made up consisting of agar 2·5 parts, Witte-peptone 1 part, salt 0·5, and meat broth 100 parts. The value was then determined of enriching this medium with each of the following substances separately : alkali albumin, serum albumin, globulin, haemoglobin, fibrinogen, protalbumose, deuteroalbumose, dysalbumose, nuclein, and legumin. The experiment was carried out in duplicate using two recently isolated meningococci ; and in a further series the value was determined of adding 1 per cent. of glucose as well. As a result it was found that the most copious growth occurred when the medium was enriched with serum albumin, serum globulin, haemoglobin, fibrinogen, and legumin respectively, and that when

the medium was enriched by any of these materials the further addition of glucose offered no advantage.

Owing to the fact that legumin was the cheapest of these enrichment materials, and readily to be obtained by extracting peaflour with saline, it was decided to define the enrichment value of this vegetable protein more closely for the present purpose. To this end, an extract of peaflour was prepared by placing 10 grammes of it in a flask with 10 grammes of salt and 100 ccm. of distilled water. The flask was then well shaken, steamed, its contents filtered, and the filtrate sterilized by steaming it on two successive days.

Using again 2·5 per cent. of agar for stiffening purposes, the nutritive value to the meningococcus was determined of a number of substances such as ordinary meat broth, the trypsinized broth of Douglas, solutions of the peptones of Armour, Witte, Savory and Moore, and of horse serum, peaflour extract, and glucose, separately and in various combinations. As a result it was found that agar dissolved in the trypsinized broth of Douglas was superior to that dissolved in ordinary meat broth, and that for purposes of enrichment the saline extract of peaflour gave better and more striking results in augmenting growth of the meningococcus than any of these other enrichment materials. A medium was thus arrived at consisting of 2·5 per cent. agar dissolved in the trypsinized broth of Douglas, to which medium when it has reached its final stage is added 5 per cent. of the saline extract of peaflour. This medium was named ' Trypagar ' (Trypsin-broth-Pea-Agar).

A comparative study was next made of the ability of this medium to grow the meningococcus from the nasopharyngeal secretion direct of a series of known carriers ; and its practical value for this purpose established. As enrichment was found to improve the growth, at first serum was sent out for this purpose, but later a saline dilution of rabbits' blood was supplied, because it proved both cheaper and more efficient than the serum. The dilution of rabbits' blood used consists of 1 part fresh blood to 19 parts of saline, and 10 per cent. of ether is added for laking purposes and also as a preservative. Two per cent. of this diluted rabbits' blood is added to the trypagar after melting it and cooling it to 50° C. By further comparative tests with the nasopharyngeal secretion of carriers it was found that the medium was most successful when it was neutral in reaction. At first phenolphthalein was used as indicator, but later at the suggestion of Major Tulloch it was found better to grade the medium against two indicators separately, namely, phenolphthalein and α-naphtholphthalein, and to adjust the reaction so that it is midway between the neutral points of these two indicators.

After April of 1915 trypagar was in continual use for cultivating the meningococcus, and gave most satisfactory results. In subculture the meningococcus grows on it profusely without enrichment. In order to determine to what particular constituent of the peaflour the stimulating actior of its extract on the meningococcus is due, Lieut.-Colonel Martin Flack carried out an investigation. He found that the legumin as such has little or nothing to do with it, the stimulating action being due chiefly to a vitamine in the pea. That

this is correct is indicated by the fact that the peaflour is apt to become inert if it is over-kilned before use, and further by the fact that trypagar may lose its power of growing the meningococcus if it is melted in the autoclave instead of in the steamer.

Three of the four requirements formulated at the beginning of this section are satisfactorily fulfilled by trypagar. It fails, however, to satisfy requirement 5. For stock cultures various media have been tried from time to time, such as ascites broth, starch medium, and egg medium. The last has been found to be far the best, and cultures may live on egg medium for as long as eight months provided the plugs are waxed. This medium is prepared by the simple expedient of breaking up the yolks of eggs with aseptic precautions, adding an equal volume of saline, and then pouring the medium into sterile tubes and inspissating them. It may here be added that Corporal J. W. Hughes, B.Sc., found that it is quite unnecessary to get an inspissator for preparing this medium. By screwing up the safety valve of the autoclave and then heating the sloped tubes in the autoclave slowly to 100° C. the medium becomes perfectly inspissated without being fissured by air bubbles.

It was found by Flack that whereas the vitamine of the pea stimulates growth of the meningococcus, there is another vitamine in the wheat germ that preserves the meningococcus alive in culture. There is no doubt that this is the explanation of the action of the starch medium of Vedder in preserving cultures of the meningococcus. The chief objection to Vedder's medium is the difficulty of sterilizing it, as, in spite of the usual steamings, an insidious and very resistant Gram-negative bacillus is apt to survive that slowly comes up and contaminates the stock cultures of meningococcus on this medium. For this reason, and also because in our hands it has given better results, egg medium has been used as a routine medium for preserving stock cultures of the meningococcus.

5. Serological Definition of the Meningococcus.

Prior to 1915 the chief Gram-negative cocci of the nasopharynx distinguishable from the meningococcus by their morphological, cultural, and fermentative characters had been identified and named, e.g. *M. catarrhalis*, *M. cinereus*, *M. siccus*, and *M. flavus I*, *II*, *III*. A group of pharyngococci, however, remained to which the work of Rautenberg, v. Lingelsheim, Flugge, Kutscher, and Lieberknecht had called attention. These pharyngococci were found to be similar to the meningococcus in the characters mentioned, and only to be differentiated from it by the agglutination test. The observations of Lieberknecht in particular had indicated that in order to perfect the differentiation of some of these pharyngococci from the meningococcus it was necessary to use the absorption test as well as mere agglutination. The work of all these observers, however, was vitiated by their failure to make an adequate determination of the degree of serological diversity among meningococci themselves. That such diversity obtains had been shown by the recent observations of Dopter who had found three different kinds of parameningococcus in addition to the meningococcus ; but as to the frequency of each

of these serological forms of the meningococci, and their individual relationship to epidemic meningitis there was no evidence.

Definition of the Meningococcus of the outbreak by the Agglutination and Absorption Test. In view of the complicated position revealed by the literature, and especially in view of the fact that no big outbreak of cerebrospinal fever had occurred in which the validity or otherwise of the later somewhat sporadic observations had been put to the test, it was obvious that before the agglutination test could be introduced as a routine measure for the purpose of identifying carriers in the recent outbreak, it was necessary to start *ab initio*, and first of all to define meningococci isolated from the patients with the agglutination test and to employ the absorption test as well.

The rationale of the absorption test is as follows. It would seem that the agglutination test, like all others, has its limits, and in particular, that unless due precautions are taken, especially when using a high-titre serum, false inferences may be drawn from its results owing to the presence in the serum of an abundance of group or co-agglutinins. In order to exclude errors from this source it is necessary when identifying a given Gram-negative coccus by the agglutination test to *prove* that agglutination when it occurs is brought about by the specific or homologous agglutinin present in the serum, and not by a co-agglutinin. Fortunately, the absorption method of Castellani enables this proof to be effected with considerable precision by the comparatively simple expedient of measuring the amount of specific agglutinin in the serum before and after saturating it with the micro-organism of test. If the specific agglutinin is removed from the serum by the test micro-organism in the same manner as by the micro-organism homologous to the serum, then their identity may be inferred ; but if after saturation with the test micro-organism the specific agglutinin is still present in undiminished quantity, clearly the agglutination of the test micro-organism originally effected by the serum must be attributed to the action of a co-agglutinin. Actual proof of this is furnished as well if after saturation, in spite of the presence of the specific agglutinin in undiminished quantity, agglutination of the test coccus no longer takes place. When conducting an investigation so responsible as the present one, therefore, the absorption test was indispensable in order to exclude errors due to the action of co-agglutinins.

Meningococci, accordingly, were collected and a systematic serological investigation was made of 32 specimens all isolated from the cerebrospinal fluid of military cases of cerebrospinal fever during the first months of the outbreak. As full details of this investigation have been published elsewhere, only its general features and results will be considered. A suspension of each coccus suitable either for injecting rabbits, or for carrying out agglutination tests was prepared by Raymond Koch's method of washing off the growth from a number of plates in saline, heating the suspension to 65° C. for 30 minutes, standardizing it, and then adding 0·5 per cent. phenol. The suspensions were standardized to a uniform turbidity equalling that of a suspension containing 2,000 million meningococci per c.c. For providing agglutinating serum, young rabbits were used as recom-

mended by Kutscher and by Elser and Huntoon. These rabbits were prepared by an intensive method worked out for the purpose. A standard procedure for performing the agglutination and absorption tests having been defined experimentally, all the agglutination and absorption tests were carried out in exactly the same way, the macroscopic method being used, and the results read off after exposing the tubes for 24 hours in an incubator at 55° C.

A rabbit was prepared against the first of the 32 meningococci and when after ten days its serum had reached a sufficient titre (1 : 400—1 : 800) for the homologous coccus, the serum was collected and all 32 meningococci tested against it. As a result 19 of the 32 meningococci were agglutinated by this serum equally as well as the homologous coccus. A second rabbit was now prepared against the first of the remaining cocci and the process repeated, with the result that 8 further meningococci were accounted for by this serum. The remaining 5 meningococci were found in the same way to provide 2 further groups, 4 belonging to one, and the last coccus comprising a group by itself.

The differentiation effected, therefore, was as follows :

Type	1	2	3	4
Specimens	19	8	4	1

Complete cross-agglutination and cross-absorption tests were carried out. They established the strict specificity of the agglutinin of each type, and they also brought out the tendency of serum prepared against Type I to contain co-agglutinin for Type III and *vice versa*, and a similar tendency of sera prepared against Types II and IV respectively to contain co-agglutinins for each other.

As soon as this serological classification of the types of meningococcus at work in the outbreak had been effected, an investigation was made of carriers who were still in isolation because they carried a micro-organism indistinguishable from the meningococcus in morphological, cultural, and fermentative characters. Out of ten such carriers examined by the staff of the Central Laboratory, one proved to be carrying a specimen of Type I meningococcus, and five carried specimens of Type II ; the remaining four carried Gram-negative cocci that could not be identified serologically with any of the four defined types of the meningococcus, and were accordingly liberated. As an additional precaution three of the nasopharyngeal cocci were injected into rabbits ; the first was a coccus serologically and in all other respects identical with Type II meningococcus. The other two were nasopharyngeal specimens that agglutinated slightly with antiserum to Type I meningococcus, but failed to absorb the specific meningococcus agglutinin from that serum. It was found that all the specimens of Type II meningococcus agglutinated with the serum prepared against the first of these nasopharyngeal cocci and removed the specific agglutinin therefrom. On the other hand, the sera prepared against the two other nasopharyngeal cocci appeared to have but slight agglutinating action on any of the types of meningococcus, and in both cases the specific agglutinin, though readily absorbed by the homologous pharyngococcus, failed to be removed by any of the types of meningococcus. Moreover,

these two nasopharyngeal cocci appeared to be serologically distinct from one another. Major Tulloch has since made an extensive study of pharyngococci resembling the meningococcus, as will be seen later (pp. 111–127).

Differentiation of the meningococci operating in the outbreak into four distinct serological types was completed by September 1915. Thereupon, Major T. G. M. Hine, after making a comparative investigation of various methods of dosage for the purpose, succeeded in elaborating an improved intensive method of preparing agglutinating serum for the meningococcus. As a result of this research Major Hine was able to add the manufacture and supply of monotypical agglutinating serum to the duties of the Supply Department of the Central Laboratory already organized by him. From that time onward the agglutination test came into general use for identifying the meningococcus, and proved to be quite indispensable for this purpose. The prevalence and frequency of the several serological types of the meningococcus throughout the outbreak and their significance will be considered later (pp. 128–132).

6. The Pathogenicity of the Meningococcus.

Notwithstanding the illuminating studies by Flexner, Dopter, and others of the interaction between the meningococcus and its host, much still remains to be explained. It is now generally agreed that during an outbreak of cerebrospinal fever, for every case in which the meningococcus succeeds in setting up meningitis there is a plurality of persons in whom it does not get further than the nasopharynx. But if as a rule the human defence is successful, there is no infection that on occasion is capable of producing death more swiftly than cerebrospinal fever. The clinical and pathological evidence adduced by Maclagan and Cooke to show that in fulminating cases of this disease adrenal injury occurs, and the encouraging results obtained by them in such cases with adrenalin, suggests an explanation of the collapse of the general resistance in at any rate some of these rapidly fatal cases ; and possibly when the mode of action of the meningococcus has been better defined, the breakdown of other factors in the human defence will be brought to light, and suitable treatment devised.

But apart altogether from variations in the defence, to what extent does the meningococcus itself vary in pathogenicity ? and on what special attributes does its pathogenic action depend ? These points are of such obvious importance that during the recent outbreak the opportunity was taken of attempting to gain a little further information concerning them. To this end, in the first place comparison was made of the virulence and toxicity respectively of a series of strains of the meningococcus for the mouse. Secondly, a search was made for evidence of the secretion of soluble toxin by the meningococcus when growing *in vitro* and also *in vivo*. Lastly, a close study was made of the meningococcus with a view to defining individual substances contained within it of importance in relation to its pathogenic action.

COMPARISON OF THE VIRULENCE AND TOXICITY OF 14 MENINGOCOCCI

Condition of Coccus.			Alive.						*Killed by* Ether.			*Killed by* Heat (55° C.).			*Remarks.*
No.	*Type.*	*Strain.*	*10,000*	*5,000*	*2,500*	*1,000*	*500*	*250*	*10,000*	*5,000*	*2,500*	*10,000*	*5,000*	*2,500*	
1	I	Littledale	+	—	—										
2	,,	Howes	+	+	—				+	—	—	—	—	—	
3	,,	Smith	+	+	—					—	—	—	—	—	
4	,,	Chalon	—	—	—				+	—	—	—	—	—	
5	,,	Jenkin	+	+	+	+	—		—	—	—	—	—	—	Recent.
6	II	Foster	—	—	—				+	—	—	—	—	—	
7	,,	Cleaver	+	—	—				—	—	—	—	—	—	
8	,,	Morgan	—	+	—				—	—	—	—	—	—	
9	,,	Brook	+	+	—				+	—	—	+	—	—	Recent.
10	,,	Pugh	+	+	+	+	+	—	—	—	—	—	—	—	Recent.
11	III	Chase	+	—	—				—	+	—	—	—	—	
12	,,	Groom	+	—	—				—	—	—	—	—	—	Recent.
13	,,	Howes	+	—	—				+	—	—	+	—	—	
14	IV	Jones	+	—	—				+	—	—	+	—	—	

Note.—The figures at the tops of the columns indicate the approximate number of million cocci injected.

+ = death of mouse. — = recovery.

Meningococci 5, 9, 10, and 12 were all injected within a few days of isolation from severe cases. The remainder had been in stock for several months before injection.

A. The Effect of Various Measures on the Pathogenicity of the Meningococcus.

(*a*) *Pathogenicity of the same Meningococcus when alive, and when killed by Ether and by Heat respectively.*

Procedure was as follows: After 16–18 hours' incubation at 37° C. the growth on plates or slopes of trypagar was suspended in broth, and after estimating the number of cocci present by means of the turbidity test, the suspension was divided into three portions. The first of these was put at once into a water bath at 37° C. for 30 minutes. The cocci in the second portion were killed by adding 10 per cent. of ether to the suspension and shaking the tube, which was then put in the water bath at 37° C. for 30 minutes to drive off the ether. The third portion was placed in a water bath at 55° C. for 30 minutes in order to kill the cocci. After a culture had been made from each of these suspensions in order to prove that the cocci were alive in case of the first, and dead in the other two, the M.L.D. of each was determined for mice intraperitoneally in the usual manner. To exclude error, all mice that died were examined and a culture made from the heart's blood. The result of an investigation of 14 meningococci in this way is shown in the table on p. 21.

This table brings out the following points:

(1) That in the majority of these meningococci the pathogenicity for the mouse was reduced when the coccus was killed either by ether or by heat.

(2) That in several instances heating appeared to reduce the pathogenicity rather more than etherization.

(3) That the difference between the pathogenicity of the coccus alive and dead, i. e. between its virulence and toxicity, was far less in case of the older cocci than in some, though not in all, of the most recently isolated ones.

(4) That the reduction of pathogenicity produced either by ether or heat was most marked in case of the two most virulent cocci of the series (Nos. 5 and 10), both of which had been isolated recently from fulminating cases of cerebrospinal fever.

(*b*) *The Effect on its Pathogenicity of suspending the living Meningococcus in broth and in distilled water respectively.*

Procedure was as follows. Two meningococci of known virulence were each cultivated for 18 hours at 37° C. on a pair of trypagar slopes of equal size. Next morning all four slopes were completely covered with growth. One slope of each meningococcus was then suspended in broth and the other in distilled water. A turbidity test indicated that in the case of each coccus the density of the suspension in broth and in water respectively was approximately the same. A portion of the broth suspension of each coccus was now killed by adding 10 per cent. of ether. After all of the suspensions had stood at 37° C. for 30 minutes, the pathogenicity of each was determined on mice. The results were as follows:

Type.	Strain.	*Coccus in Broth.*			*Do. killed by Ether.*			*Coccus in Water.*		
		0·1	*0·05*	*0·025*	*0·1*	*0·05*	*0·025*	*0·1*	*0·05*	*0·025*
I	Smith	+	—	—	—	—	—	—	—	—
II	Pugh	+	—	—	—	—	—	—	—	—

NOTE.—The doses refer to fractions of a slope culture.
+ = death of mouse. — = survival.

It would appear that etherization of the broth culture and suspension of the coccus in water are alike unfavourable to its pathogenicity. In the present experiment cultures made from the suspensions in water at the time of injection showed some growth, though less than similar cultures from the suspensions in broth. From some observations on the vitality of the meningococcus in various menstrua made later (p. 39) it would appear that the probable explanation of the lower pathogenicity of the water suspensions in this experiment was that the mortality rate of the suspended cocci was higher in the water than in the broth.

(c) *The Effect on its Pathogenicity of killing the Meningococcus by Desiccation.*

In order to determine the effect of drying the meningococcus, broth suspensions were made in the usual manner from young cultures of four meningococci on trypagar, and a series of falling doses of each measured out in duplicate in 0·5 c.c. amounts in watch glasses. In the case of each coccus the contents of one set of watch glasses were now injected into mice. The duplicate doses in the remaining watch glasses then each received a drop of ether to prevent growth of contaminations, and were placed in a desiccator which was exhausted of air and placed for one night at 37° C. Next morning the dry deposits were each suspended in 0·5 c.c. of distilled water and after a drop had been cultivated, injected into mice. The cultures remained sterile. The results on the mice were as follows :

(1) Type I, strain Chalon. Pathogenicity, alive, 5,000 million positive, 2,500 million negative. Toxicity, dried, 20,000 million negative.

(2) Type II, strain Morgan. Pathogenicity, alive, 5,000 million positive, 2,500 million negative. Toxicity, dried, 5,000 million positive, 2,500 million negative. In view of this unusual result, the experiment was repeated, but the result was the same.

(3) Type II, strain Caruna. This coccus had been recently isolated from a very severe case that died in a few days. The pathogenicity of the coccus when alive was 500 million positive, 250 million negative. The toxicity of this same coccus when dried was 4,000 million positive, 2,000 million negative.

(4) Type II, strain Sister. This coccus had also been isolated from a recent case in which the patient recovered. The pathogenicity alive was 2,000 million positive, 1,000 million negative. When dried, a dose of 4,000 million was negative.

In the case of three of these four meningococci, therefore, desiccation of the coccus reduced its pathogenicity very materially. This was especially well marked in the case of No. 3, the most virulent of the four cocci.

(d) The Effect of Age on the Pathogenicity of the Meningococcus.

The particular meningococcus (Lindenbaum) used in this experiment had recently been isolated from a specimen of cerebrospinal fluid which was kindly given to me by Colonel Flexner when in this country. The case from which it came succumbed rapidly, and the coccus, which was swarming in the cerebrospinal fluid and in pure culture there, proved to be a specimen of Type II. Six equal-sized slopes of trypagar were inoculated from an early subculture of it and placed at 55° C. After one day the growth on two of the slopes was suspended in broth, thoroughly mixed, and divided into two portions, one of which was then heated for half an hour to 55° C. After a culture had been made from each portion, the pathogenicity for the mouse was determined of the coccus when alive, and when killed by exposure to 55° C. With the remaining slopes the test was repeated after they had been in the incubator for three and six days. As regards vitality, it was found that the suspensions before being heated to 55° C. gave profuse growth when the coccus was 1 day old ; and about half as much growth when it was three days old ; but no growth when it was six days old. In all of the heated suspensions the coccus was dead. The result of the pathogenicity tests was as follows :

Condition of Meningococcus.	*Dose (fraction of slope).*	*Age of Meningococcus.*		
		1 *day.*	3 *days.*	6 *days.*
Unheated :	0·2	+	+	+
	0·1	+	+	—
	0·05	+	—	—
	0·025	+	—	—
Heated to 55° C. :	0·2	—	—	+
	0·1	—	—	—
	0·05	—	—	—
	0·025	—	—	—

+ = mouse died. — = mouse survived.

The outcome of this experiment is instructive. Corresponding with its vitality, the pathogenicity of the unheated coccus was high on the first day when $\frac{1}{40}$ of a slope proved fatal. It had decreased by 75 per cent. on the third day, and by 50 per cent. further on the sixth. In case of the heated coccus the zone of pathogenicity was not reached either on the first or third day, but on the sixth its pathogenicity had apparently increased and was now equal to that of the unheated coccus. As by the sixth day on the trypagar slope the coccus had died, its pathogenicity for the mouse was no longer affected by heating it to 55° C. for 30 minutes. From experiments to be described later it seems probable that the increase in toxicity shown by the heated coccus on the sixth day as compared with the result on the first and third day was due to liberation of its endotoxin by autolysis.

(e) Conclusion.

The preceding observations indicate that the most pathogenic examples of the meningococcus for the mouse are to be found in

certain specimens of it recently isolated from severe or fulminating cases of cerebrospinal fever. The major portion of the pathogenicity of these very virulent meningococci is labile, and would appear to be closely associated with the ability of the coccus to multiply actively in the tissues of its host ; since mice succumbing to these virulent cocci invariably gave a profuse growth of the coccus from their blood.

On the other hand, even when dead the meningococcus still possesses considerable pathogenic power ; and this toxicity would seem to be of a more stable character. The result of further study of this endotoxin of the meningococcus will be described later.

B. Search for evidence of the Secretion of a Soluble Toxin by the Meningococcus.

From the observations just described it would appear that the major portion of the pathogenicity of certain recently isolated meningococci for the mouse depends upon a factor that is thermolabile, etherlabile, and that disappears from a culture on trypagar in the course of a few days as the coccus dies. One explanation is that the factor in question consists of the vital capacity of the coccus to multiply. But clearly this cannot be the whole explanation because of the very great difference between the pathogenicity for the mouse of various meningococci equally able to multiply *in vitro*, to judge by their profuse growth on trypagar. The question therefore arises : Is the pathogenicity of these virulent meningococci due to their ability to secrete a toxin ?

Provided that such a toxin exists, it may conceivably be either intracellular or extracellular. It is necessary, therefore, in the first place to see if any evidence can be found of the secretion of an extracellular toxin by the meningococcus.

(*a*) *Search for Extracellular Toxin in Cultures in vitro.*

On a number of occasions cultures of virulent strains of the meningococcus were made in fluid media consisting of broth enriched by ascites fluid, serum, or rabbits' blood. After incubation for periods varying from a few days to as many weeks the cocci were separated off by centrifuge or by filter and the pathogenicity of the fluid tested by injecting amounts up to 0·5 c.c. of it into mice intraperitoneally. In no case was any certain evidence obtained of the presence of soluble toxin in the medium. On the other hand the centrifugal deposit containing the separated cocci when tested in the same way gave distinct evidence of pathogenicity. Such toxin therefore as was demonstrable was retained within the bodies of the cocci.

(*b*) *Search for Extracellular Toxin in Cultures in vivo.*

It seemed possible that the failure to find toxin in cultures *in vitro* might be due to unsuitability of the medium. It was decided, therefore, to repeat the test in the animal body, and to see if any evidence could be obtained of the secretion of an extracellular toxin by the meningococcus in the peritoneal cavity of the guinea-pig.

The same meningococcus (Lindenbaum) was used in this experiment as had been employed in the test in which the effect of age on

the pathogenicity of the meningococcus was determined. Two guinea-pigs were selected, each of which weighed approximately 400 grammes. The approximate M.L.D. for a guinea-pig of this size of the coccus here employed was found to be about 50,000 million cocci. One of the guinea-pigs received intraperitoneally 2·5 c.c. of Type II antimeningococcus serum, and one hour later both pigs received a dose consisting of 400,000 million living cocci intraperitoneally. The next day both guinea-pigs had succumbed.

Guinea-pig No. 1. This animal had received no serum. It showed a large number of minute subcutaneous haemorrhages giving these tissues a 'sooty' appearance. The peritoneal exudate contained much blood, and a film of it showed microscopically swarms of meningococci and fairly numerous red blood corpuscles, but no white corpuscles. About 10 c.c. of the exudate was collected without difficulty and centrifuged hard until it was quite clear; the red blood corpuscles then forming a layer 0·5 c.m. thick at the foot of the tube. The absence of free haemoglobin in the clear fluid showed that in spite of the escape of blood into the peritoneal cavity little or no haemolysis had occurred there; 0·5, 0·2, and 0·1 c.c. of this clear fluid was cultivated on trypagar slopes and similar amounts of it were injected intraperitoneally into mice. The cultures all grew the meningococcus mixed with *Streptococcus faecalis*. The mice were slightly ill for one day, but had quite recovered in 48 hours.

Guinea-pig No. 2 had received intraperitoneally 2·5 c.c. of Type II antimeningococcus serum one hour before injection of the coccus. The subcutaneous tissues showed no visible changes. The peritoneal exudate was straw coloured with a few flakes of fibrin and leucocytes; it contained no blood. About 10 c.c. of the exudate was collected. The film of this showed microscopically comparatively few particles of any kind. Here and there were a few shadows of cocci, and some polymorphonuclear cells. The fluid was centrifuged hard for three hours and then cultures were made from 0·5, 0·2, and 0·1 c.c. of it, and similar quantities were injected into mice. The meningococcus grew in all of these cultures in pure culture. The mouse that had received the largest dose was ill next day but had recovered in 48 hours: the other mice were unaffected.

No convincing evidence, therefore, was obtained of the presence of free toxin in the peritoneal fluid of either of these animals. As it seemed possible, however, that such toxin might have been present and perhaps have been fixed by the tissues during the interval that elapsed between the death of the animal and the post-mortem examination, the experiment was repeated and the peritoneal exudate removed for examination before the animal had succumbed to the injection.

Guinea-pig No. 3. This animal, which weighed 390 grammes, received intraperitoneally 400,000 million living meningococci at 9 a.m. and five hours later it was killed by ether and the peritoneal exudate collected. The exudate was turbid and showed a few red blood cells. A film of it revealed swarms of meningococci, but nothing else. Cultures showed that living meningococci were present in this exudate to over 1,000, but less than 10,000 per c.c., while the blood of the animal showed over 100 but less than 1,000 living meningococci per c.c.

The following tests were made with the exudate: 0·1, 0·01, and 0·001 c.c. of it were injected intraperitoneally into mice with the result that the mouse injected with 0·1 c.c. died in three days, the others recovered. The rest of the exudate was centrifuged hard and 0·5, 0·2, and 0·1 c.c. of the supernatant fluid injected into the peritoneal cavities of mice which, however, failed to develop any illness.

So far as they went, therefore, these experiments failed to show any certain evidence of free toxin capable of affecting mice in the peritoneal fluid of the guinea-pig in which the meningococcus was present in living condition and in large numbers. It is not justifiable, of course, on this evidence to deny the possible presence of free toxin there for the guinea-pig, and it is regrettable that a dearth of young guinea-pigs prevented the toxicity of the exudate being tested on animals of the homologous species. But while it is not claimed that these observations established the absence of free toxin, they certainly failed to provide any certain evidence of its presence.

There remains the possibility that the high virulence of certain recently isolated meningococci may be due to their possessing an intracellular labile toxin; or it is conceivable that they may possess an actively autolytic tendency that renders their endotoxin more soluble in body fluids than is the case with less virulent cocci. Flexner has emphasized the importance of this autolytic tendency of the coccus in setting free its endotoxin *in vivo*, and it is possible that some factor at present undefined inhibited its demonstration in the experiments described above.

7. Intracellular Constituents of the Meningococcus of pathogenic importance.

The preceding investigations having failed to provide any sure evidence of the presence of soluble toxin in the fluid surrounding the meningococcus when growing either *in vitro* or *in vivo*, an attempt was made to identify intracellular constituents of this micro-organism of importance from the point of view of its pathogenic action. So far as this investigation has progressed, three components of the meningococcus of pathogenic interest have been identified and studied, namely, a haemolytic substance, a reducing agent, and a powerful endotoxin. The information which has been obtained with regard to each of these intracellular constituents of the meningococcus respectively will now be described.

A. Studies of an Intracellular Haemolytic Substance contained by the Meningococcus.

During the recent outbreak of cerebrospinal fever among the military forces a special endeavour was made to define the particular qualities that determine the potency of antimeningococcus serum. In the course of this inquiry it became necessary to ascertain the ability of individual specimens of serum to neutralize the endotoxin of the meningococcus. In order to obtain endotoxin for this purpose trial was made of the method introduced by Besredka and used successfully by himself and others for extracting endotoxin when determining the antiendotoxic capacity of various antibacterial sera.

Although Besredka's method does not appear to have been applied previously to the meningococcus, it has been found with a few minor modifications to be of value also for the purpose of preparing the endotoxin of this micro-organism. Procedure is as follows: A recently isolated meningococcus of known serological type, and of good virulence for the mouse intraperitoneally is sown on fifty or more plates of trypagar. After 18 hours' incubation at 37° C. the growth is removed from these plates by suspending it in saline, and this suspension, after microscopical examination, is then centrifuged hard for 2 hours or more until the cocci are deposited. The bulk of the supernatant saline is then thrown away and the deposited cocci transferred to a glass dish ; a few drops of ether are added as a preservative, and the dish placed over sulphuric acid in a desiccator which is then closed, exhausted of air, and placed at 37° C. The dried deposit on the plate next morning is scraped off, powdered, and examined microscopically: it consists entirely of the dead and dried bodies of meningococci. The M.L.D. of this powder for the mouse intraperitoneally has been found to average from 2–4 milligrammes, the degree of toxicity depending apparently on the extent to which the toxin within the bodies of the cocci can be got into solution before injecting it. From some careful weighing experiments kindly carried out for me by Corporal J. W. Hughes, B.Sc., R.A.M.C., it has been estimated that one milligramme of the dried meningococcus represents the bodies of about 7,000 million raw meningococci.

On triturating this powder by pounding it in an agate mortar, at the same time slowly adding distilled water, and by then making some haemolytic tests with this material it was found that the meningococcus contains a substance possessed of lytic properties for the red blood-cells of man, and of the rabbit, guinea-pig, and mouse. In a paper published in the *British Medical Journal* of January 26, 1918, mention was made of the fact that the dried bodies of meningococci of all four serological types are haemolytic ; and that this property was not lost on heating them for 30 minutes to 60° C.

As this haemolytic substance appeared to be retained within the body of the meningococcus as tenaciously as is the endotoxin, but unlike the latter, the lytic substance is readily demonstrable *in vitro*, it has been investigated at some length with a view to ascertaining the conditions under which it is liberated from the interior of the coccus, and its general properties. Not only was there reason to suspect that the former might possibly be a guide to the conditions under which the endotoxin also is set free, but an equally important point demanding investigation at that time was the question as to whether the haemolysing agent is or is not identical with the intracellular toxin of the meningococcus. In order to make the inquiry complete an investigation was made in the first place of the haemolysing powers of the living meningococcus when growing in culture.

(*a*) *Haemolytic Action of the Meningococcus when growing in Culture.*

Experiment 1. Blood was drawn aseptically from the finger and transferred to the condensation fluid of a dozen trypagar slopes which were then inoculated with the meningococcus and incubated at 37° C. Although profuse growth occurred in all of the tubes none of them

showed any evidence of haemolysis until between the fourth and seventh day. Blood placed in the condensation fluid of a control uninoculated tube kept with these others did not haemolyse until the tenth day. In some confirmatory experiments made later with other batches of medium haemolysis occurred somewhat earlier, beginning on the second or third day.

Experiment 2. Four of the most actively lytic meningococci in the first experiment were each sown on to two slopes, and blood was placed in the condensation fluid of one, but not into that of the other. All of the tubes were incubated at 37° C., and when after the lapse of several days haemolysis was evident in the tubes containing blood, fresh blood was introduced into the condensation fluid of the duplicate tubes which were then returned to the incubator. Well-marked haemolysis was produced in all in a night. This experiment was repeated on two further occasions with a similar result.

The inference drawn from these observations was that when the meningococcus is growing on trypagar slopes at 37° C. the haemolytic substance contained within it is retained until after two or more days, when it is liberated whether blood is present or not. The accuracy of this inference was now tested by comparing the haemolytic power of a suspension of the same meningococcus after it had been grown for one day and seven days respectively on trypagar.

Technique. The method employed in the following haemolytic tests was as follows : Small test tubes, 3 in. long and ½ in. broad, were used. These were sterilized, plugged with wool, and were arranged in metal racks fitting into a water bath kept at 37° C. The procedure followed was to add 0·1 c.c. of a 5 per cent. suspension of washed human red blood-cells to 0·5 c.c. amounts of falling dilutions of a suspension of meningococcus made by suspending the growth of a slope or plate in 0·85 per cent. saline, a control of the corpuscles and saline alone being always used as well. After exposure for 2 hours in a water bath at 37° C. the racks holding the tubes were taken out, allowed to stand on the laboratory bench, and read off next morning. In comparing the haemolytic power of various meningococci, the procedure used in many of the later experiments was to suspend the whole of the surface growth of an 18-hours' slope culture in 1 c.c. of saline after removing the condensation fluid. Taking the average number of meningococci present in such a suspension as 20,000 million, dilutions were prepared from it containing 10,000 million, 5,000 million, 1,000 million, &c., in 0·5 c.c. When working with plate cultures the average total growth of a completely covered trypagar plate has been taken as 200,000 million cocci, and dilutions made in a similar manner as in the case of slopes. By proceeding in this way a uniform method was arrived at of testing the haemolytic power of a meningococcus. Precisely the same procedure was employed later when investigating the reducing power of the meningococcus.

It is advisable to carry out all of these tests as aseptically as possible. Unless this is done errors are apt to creep in, particularly from the action of the skin staphylococcus—a hardy micro-organism that grows readily in saline suspensions of the meningococcus, and has a well-marked haemolytic action. For this reason, it is always advisable to scrutinize very carefully with Gram's stain any suspension of

meningococci that have been kept for more than a day ; unless of course the suspensions have been heated, or some suitable antiseptic added.

(*b*) *Influence of Age on the Haemolytic Power of the Meningococcus.*

Seven meningococci were subcultured on to trypagar slopes and a comparison made of the haemolytic power of each after incubation for one day and seven days respectively at 37° C. The growth of each culture was suspended in 1 c.c. of saline and a series of dilutions made of 0·5 c.c. of this suspension through four successive 0·5 c.c. amounts of saline. The haemolytic power of these suspensions was then tested on washed corpuscles in the manner described. The result was as follows. When one day old all seven meningococci failed to produce any haemolysis in a dose of $\frac{1}{8}$ of a slope representing approximately 2,500 million cocci, and $\frac{1}{4}$ of a slope only produced a trace of haemolysis. When seven days old, on the other hand, the haemolytic effect was well marked in case of all the cocci even in $\frac{1}{32}$ of a slope, representing approximately 625 million cocci. Haemolytic power therefore was over four times as strong in all the cocci on the seventh day as on the first. Subcultures showed that all the cocci were alive on the first day, and dead on the seventh. Clearly then, although dead, the meningococcus is far more haemolytic in a seven-days-old culture on trypagar than in a young actively growing culture on this medium.

(*c*) *Diffusibility of the Haemolytic Substance.*

Three ten-weeks-old cultures of the meningococcus in broth, and in the same medium enriched by serum and blood respectively, were now examined in order to see if the haemolytic agent could be detected in the fluid of the culture. The cultures were centrifuged until clear and the haemolytic power of the fluid compared with that of the deposit. No haemolytic action was seen in case of the fluid when diluted 1 : 10 of saline ; but the deposit containing bodies of meningococci produced definite haemolysis in all three cases in a dilution of 1 : 10, and in two of them up to a dilution of 1 : 40. The haemolytic substance, therefore, had not become diffused through the medium, but was bound up with the bodies of the cocci.

(*d*) *Effect of Autolysis in setting free the Haemolytic Substance.*

The principle adopted was to employ the young coccus and to determine the influence of autolysis upon its haemolytic activity.

Effect of the Autolysin. It seemed probable that the autolytic enzyme which Flexner showed to be such an important factor in the physiology of the meningococcus would have an important influence on the liberation of the haemolytic substance. As the autolysin is greatly diminished by heating the coccus to 65° C. for 30 minutes, a fresh saline suspension of the raw coccus was divided into three parts. One of these portions was left alone, the other two heated for half an hour to 55 and 65° C. respectively. The three suspensions were then kept for 10 days at 37° C., after which their haemolytic power was compared. It was found that the unheated coccus gave best results ; then the coccus heated to 55° C., and lastly the coccus

heated to 65° C. The autolysin therefore appears to have a distinct influence in liberating the haemolytic substance.

Effect of various factors on Autolysis for setting free the Haemolytic Substance. (1) As a preservative for the suspensions of the meningococcus toluol was found to be far superior to phenol. (2) As regards macerating fluids saline appeared to be better than either water or broth. (3) In a comparison of dry *v.* wet autolysis (i. e. autolysis on a trypagar slope as compared with autolysis in saline) the values were about equal—perhaps slightly in favour of the dry autolysis.

(*e*) *Effect of Heat on the Haemolytic Substance.*

In the first place an actively-haemolytic suspension of the meningococcus was prepared by suspending in saline the growth from some plate cultures of it that had stood in the incubator for a week. The effect on the haemolytic activity of this suspension was then determined of heating it for 30 minutes to 55° C., 65° C., and 100° C. respectively. So far from reducing the haemolytic power, the heating appeared to improve it, haemolysis being best in the suspension heated to 100° C. The investigation was continued, using a suspension of the coccus when one day old, two days, and three days respectively ; and heating each of them to 100° C. and to 120° C. for 30 minutes. The result of this experiment proved very instructive. The raw coccus when one day old failed to haemolyse, but after being heated to 100° C. the same suspension was found to possess a distinctly haemolytic action which was increased by heating it to 120° C. In case of the coccus when two and three days old haemolysis was active before the coccus was heated; but in both cases the haemolytic power was increased by heating; the suspension heated to 120° C. being the best. The effect was now determined of heating a suspension of the one-day-old coccus to 120° C. in the autoclave for one hour, two hours, and three hours respectively. The haemolytic power of the suspension before and after being heated was as follows :

	No. of million cocci.				
Material.	*10,000*	*5,000*	*2,500*	*1,250*	*625*
Meningococcus 1 day old, unheated	+	(+)	—	—	—
Do. after heating to 120° C. for 1 hr.	+	+	+	(+)	—
,, ,, ,, 2 hrs.	+	+	+	+	—
,, ,, ,, 3 hrs.	+	+	+	+	(+)

+ = well-marked haemolysis. (+) = slight haemolysis.

It is clear from the above results that the longer the coccus was autoclaved the more its haemolytic substance was liberated. It may be mentioned that after being autoclaved at 120° C. the suspension of the young coccus became more transparent, and though microscopically the outlines of the cocci were still visible, it appeared that the meningococci had undergone partial dissolution. This is the probable explanation of the direct relation between the length of exposure in the autoclave and the haemolytic power of the suspension.

(*f*) *Effect of Desiccation on the Haemolytic Substance.*

Since the haemolytic substance is present in the dried coccus, it was plain that it withstands desiccation. An experiment, however,

was made in order to see what loss, if any, occurs during the process. A saline suspension containing 20,000 million two-days-old meningococci per cubic centimetre was divided into three portions. The first of these was heated to 55° C. for 30 minutes and then remained at room temperature over night. The second portion was dried *in vacuo* at 37° C. over night and then resuspended in the same amount of distilled water. The third portion was autoclaved at 120° C. for 30 minutes, and then divided into two parts, one of which was allowed to stand at room temperature over night, the other was dried *in vacuo* at 37° C. over night and resuspended in the same amount of distilled water next day. The haemolytic power of these four suspensions was as follows :

		No. of million cocci.					
No.	*Material.*	*10,000*	*5,000*	*2,500*	*1,250*	*625*	*312*
1	Coccus heated to 55° C.	+	+	+	(+)	—	—
2	Do. dried and resuspended	+	+	+	+	(+)	—
3	Coccus autoclaved	+	+	+	+	(+)	—
4	Do. dried and resuspended	+	+	+	+	(+)	—

Desiccation, therefore, so far from impairing the haemolytic substance, rendered its haemolytic power more prominent in case of the coccus heated to 55° C., while it failed to make any difference in the autoclaved coccus. The probable explanation of the increase in case of No. 2 above is that the dried coccus was partially broken up when resuspending it.

(*g*) *Effect of Solvents on the Haemolytic Substance.*

The next step was to determine the value of each of five solvents, namely, distilled water, saline, absolute alcohol, acetone, and ether respectively for extracting this haemolytic substance from the meningococcus. Trials have been made of each of these solvents on the meningococcus when one day old and when six days old respectively. In both series also the effect was determined of autoclaving the coccus for two hours at 120° C. before submitting it to extraction.

The procedure followed was to make a thick suspension of the coccus from trypagar plates. For this purpose 5 c.c. of distilled water was used to the plate, giving an average density of approximately 40,000 million cocci per c.c. This suspension was next divided into five equal portions of 2 c.c. The first portion was left alone, being used for a control. To the second 5 c.c. of water was added, to the third 5 c.c. of saline, and to the others 5 c.c. of alcohol, acetone, and ether respectively. After thorough admixture, the saline and water were cleared of cocci by centrifuge. In the case of the alcohol, acetone, and ether extracts, the mixture was boiled for a few minutes in a water bath and then allowed to stand for half an hour in cold water, by which time the cocci had settled to the bottom. The fluids were now removed by pipette and evaporated to dryness in watch glasses. Next day the deposits on the watch glasses were each dissolved in 1 c.c. of saline and the haemolytic power of the solution tested in the ordinary way. Controls done in the same way with the evaporated residue of 5 c.c. of each of the solvents alone proved quite negative.

Effect of Solvents on the Meningococcus when one day old. It was found that none of the five solvents had any value for extracting the haemolytic substance from the one-day-old coccus when unheated; but when this same suspension had been autoclaved for one hour at 120° C. the acetone and the alcohol both succeeded in extracting the haemolytic substance, the acetone being the more successful of the two. Water, saline, and ether all failed to extract the haemolytic substance even when the suspension had been autoclaved. It was noticed that whereas the alcoholic extract of the coccus before it had been autoclaved was quite clear, the similar extract of the coccus after it had been autoclaved was distinctly turbid from the presence therein of the haemolytic substance.

Effect of Solvents on the Meningococcus when six days old. In this case both acetone and alcohol extracted the haemolytic substance before the coccus was autoclaved, although more of the haemolytic substance was taken up by both solvents from the same suspension after it had been autoclaved for one hour at 120° C.

The haemolytic substance contained by the meningococcus, therefore, is soluble in acetone and in absolute alcohol, and when thus extracted and the solvent evaporated off, it can readily be redissolved in saline. Autoclaving the coccus at 120° C. before extracting it considerably increases the amount of haemolytic substance taken up by either acetone or alcohol. In ether, on the other hand, the haemolytic substance would appear to be insoluble: even the autoclaved coccus giving negative results with this method.

Summary. The meningococcus contains a haemolytic substance which is hidden within the young actively growing coccus, but becomes active as the coccus ages. The autolysin of the meningococcus appears to have an influence in 'unlocking' this haemolytic substance as the coccus becomes older; but though more active in the older cocci, the haemolytic substance appears to remain attached to their bodies.

The intracellular haemolytic substance of the meningococcus is very resistant to heat, and this property can be applied to demonstrate its presence in a suspension of young meningococci by the simple expedient of autoclaving the suspension at a temperature of 120° C.; by which treatment the cocci are partially disintegrated and the haemolytic substance unmasked.

This haemolytic substance is unaffected by desiccation. It is soluble in acetone and alcohol, but not in ether. When the haemolytic substance is extracted from the meningococcus by acetone or alcohol, and these solvents are evaporated off, it is readily soluble in water or in saline.

B. Studies of an Intracellular Reducing Agent contained by the Meningococcus.

While carrying out the haemolytic experiments described in the preceding section it was noticed that when washed red blood corpuscles are added to a suspension of living meningococci, and the mixture placed in a water-bath at 37° C., the bright scarlet tint of the corpuscles rapidly becomes changed to a dark purple colour. As it seemed possible that this change of colour was due to a reducing

action on the part of the living meningococcus, the experiment was repeated, using methylene blue as an indicator in place of the red corpuscles. The methylene blue quickly became bleached by the coccus, the true colour returning temporarily on shaking the tubes well so as to aerate them. Clearly then the living coccus exerts a strong reducing action on its immediate environment, and since this property of the meningococcus appears to have escaped attention hitherto, and was of considerable interest in its possible relation to the pathogenic action of the micro-organism, it has been investigated at some length.

Method. The procedure followed when studying the reducing action of the meningococcus was similar to that employed when investigating the haemolytic power of this micro-organism. Once again, the condensation fluid of a slope culture of the coccus on trypagar having been removed, the whole of the growth was suspended in the diluent ; and taking the average number of cocci contained by such a suspension as 20,000 million, falling dilutions were made as before, each bulking to 0·5 c.c. The same small tubes plugged with wool and held by racks fitting into the water-bath at 37° C. were used in both investigations. When in some of the experiments red blood corpuscles were used as an indicator, 0·1 c.c. of a 5 per cent. suspension of washed human red blood corpuscles was added to each of the tubes containing falling amounts of the suspension of the coccus. In the majority of the experiments, however, methylene blue was used as indicator because of its convenience. For this purpose a stock solution was made up of 1 : 1,000 methylene blue in distilled water, and for each test a 1 : 10 dilution was made up from the stock bottle ; and 0·1 c.c. of this dilution added to each of the tubes containing 0·5 c.c. dilutions of the suspension of the coccus. The concentration of methylene blue in each test therefore was 1 : 60,000. After adding the indicator, the tubes were well shaken to distribute it, and the racks holding them then placed in a water-bath at 37° C. Unless stated otherwise, the time of exposure was 2 hours at 37° C., although as a rule the main features of the result are clearly visible within 30 minutes.

(*a*) *Comparison of Red Blood Corpuscles and Methylene Blue as Indicators of the Reducing Action of the Meningococcus.*

Two trypagar slope cultures were made of each of three meningococci, and after 18 hours' incubation at 37° C. the growth of the pair of cultures of each coccus was suspended in saline, mixed, and falling dilutions made therefrom in duplicate series. Washed red blood corpuscles were then added to one set of dilutions of each coccus, and methylene blue to the other. The results were as follows :

			Approx. No. of million cocci present.				
Type.	*Strain.*	*Indicator.*	*10,000*	*5,000*	*2,500*	*1,250*	*625*
I	Littledale	Corpuscles	+	+	+	+	+
,,	,,	M. Blue	+	+	+	—	—
I	Smith	Corpuscles	+	+	+	+	+
,,	,,	M. Blue	+	+	+	+	—
II	Cleaver	Corpuscles	+	+	+	+	—
,,	,,	M. Blue	+	+	+	—	—

+ = reduction produced.

Although in case of all three cocci the red blood corpuscles proved the more delicate index, methylene blue was adopted for routine purposes because of its greater convenience.

(*b*) *Effect of Menstruum on the Reducing Power of the Meningococcus.*

Some preliminary experiments having indicated that the reducing power of the meningococcus is more active when it is suspended in broth than in either saline or distilled water, an experiment (see p. 37, Experiment (*b*)) was made in order to compare specimens of meningococci of all four serological types in this respect.

The reducing action, therefore, of all four meningococci was more intense in broth than in saline or distilled water.

In a further experiment the reducing power of the first of the preceding meningococci (Smith) was compared in water, saline, broth, serum (horse), and in two samples of cerebrospinal fluid, one of which was a specimen of normal C.S. fluid, while the other came from a case of streptococcal meningitis. Before use both C.S. fluids were freed of suspended matter by centrifuge. Six 18-hours equal-sized slope cultures were made of the meningococcus, and the growth on each slope was then suspended in one of the fluids referred to and a set of falling dilutions made in 0·5 c.c. amount. The reduction effected by the coccus in each menstruum is seen in the following table :

Dilutions of a 1-day Slope Culture of Meningococcus.

Menstruum.	*1 : 5*	*1 : 10*	*1 : 25*	*1 : 50*	*1 : 100*	*1 : 200*	*1 : 400*
Water . . .	—	—	—	—	—	—	—
Saline . . .	+	—	—	—	—	—	—
Broth . . .	+	+	+	+	—	—	—
Serum . . .	+	+	+	+	+	—	—
C.S. fluid (1) .	+	+	+	+	—	—	—
C.S. fluid (2) .	+	+	+	+	—	—	—

+ = reduction of M. Blue.

The reducing power of the coccus, therefore, was best in serum, then in broth, and in both specimens of C.S. fluid. As before, both saline and water were unfavourable. The explanation will appear later (cf. (*g*) below).

(*c*) *Is the Reducing Agent soluble in the Menstruum or confined to the body of the Meningococcus ?*

Several experiments were made in which the living meningococcus fresh from an 18 hours' slope was suspended in saline or broth, and then a portion of the suspension was centrifuged till clear, and the fluid separated off from the deposited cocci and tested for reducing power. Although in all these tests the suspension before being centrifuged, and also the cocci deposited from it, reduced methylene blue, no reduction was effected by the fluid washing of the cocci. In an apparent exception where the fluid of a broth suspension produced some slight reduction after being centrifuged, it was found on examination to still contain living meningococci.

Some experiments were now carried out in which rich suspensions were made of two specimens of the meningococcus in water, saline, and broth respectively, and after being kept for ten days at 37° C. the cocci were deposited by centrifuge, and the reducing power of the fluid and deposited cocci tested separately. Although the deposits produced some reduction, all the fluids were negative. Up to the present, therefore, no certain evidence has been obtained of the secretion of a soluble reducing agent by the meningococcus in cultures. It would seem, then, that the reducing property is mainly confined to the surface and interior of the coccus. The question now arises: Is this intracellular reducing agent a function solely of the living protoplasm of the coccus, or does it continue when the coccus is dead?

(d) Effect of Heat on the Reducing Power of the Meningococcus.

(i) *Result of Heating the Meningococcus to 55° C. for 30 minutes.* Numerous experiments having proved that the meningococcus is killed by exposing it for half an hour to 55° C., the effect of such exposure on the reducing power of the coccus was determined.

Two meningococci were employed in this test and the experiment was carried out in duplicate, using washed red blood corpuscles as indicator in the first series, and methylene blue in the second. The menstruum used was broth.

The results were as follows:

				Approximate No. of million cocci.					
Type.	*Strain.*	*Condition.*	*Indicator.*	*10,000*	*5,000*	*2,500*	*1,250*	*625*	*Vitality.*
I	Littledale	Unheated	R.B.C.	+	+	+	+	+	+
„	„	Heated to 55	„	+	+	+	—	—	—
II	Cleaver	Unheated	„	+	+	+	+	—	+
„	„	Heated to 55	„	+	+	—	—	—	—
I	Littledale	Unheated	M. Blue	+	+	+	—	—	+
„	„	Heated to 55	„	+	+	+	—	—	—
II	Cleaver	Unheated	„	+	+	+	—	—	+
„	„	Heated to 55	„	+	+	(+)	—	—	—

It is clear that although the exposure to 55° C. killed the coccus in all cases, it failed to abolish the reducing action. The more sensitive red corpuscles also detected a diminution in the reducing power consequent on the heating that the coarser methylene blue failed to reveal.

(ii) *Effect of Heating the Meningococcus to 60° C., 65° C., and 70° C. respectively for 30 minutes and for 1 hour.* Meningococci representative of all four types were now submitted to the above temperatures for the times stated. In all cases cultures proved that the cocci were killed by the heating. The indicator used was methylene blue. It was found that a temperature of 60° C. for 30 minutes had but little effect on the reducing activity of the cocci; and even 1 hour at this temperature, while it diminished the reducing power of the weaker cocci, had but small effect on the more actively reducing ones; 65° C. for 30 minutes was not much if at all more effective, but in 1 hour produced considerable diminution; 70° C. for 30 minutes produced very marked diminution, and in 1 hour abolished the reducing power of five out of the six cocci tested, and almost abolished it in the excep-

Experiment (*b*).

Type	*I.*				*II.*				*III.*				*IV.*			
Strain	*Smith.*				*Morgan.*				*Chase.*				*Jones.*			
Millions of cocci	*2,500*	*1,250*	*625*	*312*	*2,500*	*1,250*	*625*	*312*	*2,500*	*1,250*	*625*	*312*	*2,500*	*1,250*	*625*	*312*
Water	+	+	—	—	+	+	—	—	+	+	+	—	+	+	(+)	—
Saline	+	+	—	—	+	+	—	—	+	+	(+)	—	+	+	(+)	—
Broth	+	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+

\+ = reduction of M. Blue.

Experiment (*e*)

Character	*Reduction.*								*Haemolysis.*							
Age of coccus	*1 day.*				*8 days.*				*1 day.*				*8 days.*			
Dose (millions)	*5,000*	*2,500*	*1,250*	*625*	*5,000*	*2,500*	*1,250*	*625*	*5,000*	*2,500*	*1,250*	*625*	*5,000*	*2,500*	*1,250*	*625*
Type I. Smith	+	+	+	+	—	—	—	—	(+)	—	—	—	+	+	+	+
„ I. Howes	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	(+)
„ I. Jenkin	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	+
„ II. Morgan	+	+	+	+	—	—	—	—	(+)	—	—	—	+	+	+	+
„ II. Pugh	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	(+)
„ III. Chase	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	+
„ IV. Jones	+	+	+	—	—	—	—	—	(+)	—	—	—	+	+	+	+

tion which still showed a trace of reduction in a concentration of 5,000 million after being heated.

Inference. The reducing agent contained by the meningococcus survives temperatures between 55 and 65° C. that are fatal to the coccus, and is therefore independent of its living protoplasm. This reducing agent, however, is destroyed by exposing the coccus for 1 hour to 70° C.

At 100° C. the reducing power of the meningococcus is destroyed at once.

(*e*) *Effect of Age on the Reducing Power of the Meningococcus.*

(i) *Comparison between Meningococci aged* 1 *day and* 8 *days respectively.* An investigation was made at the same time of the reducing and haemolytic powers of seven meningococci after 1 day and 8 days' growth on trypagar respectively. Red blood corpuscles were used as the indicator of both characters. The medium used was saline. The results were as shown on p. 37, Experiment (*e*).

The effect of age on the reducing power of the meningococcus is the exact opposite, therefore, to the effect on its haemolytic power. The young living culture reduces, but does not lyse ; whereas the older dead culture lyses, but does not reduce.

(ii) *Comparison of the Vitality and Reducing Power of Meningococci between the first and fifth day.* An investigation was now made of cultures of meningococci of one to four days' growth on trypagar in order to determine at the same time their vitality and reducing power. To complete the series, some of the cocci after remaining at 37° C. for a day were then kept at room temperature and examined in the same way. The result of these observations was as follows :

				Vitality.	*Reducing Power.*			
Type.	*Strain.*	*Temp. of Growth.*	*Age.*	*(5,000)*	*5,000*	*2,500*	*1,250*	*625*
I	Smith	37° C.	1 day	+	+	+	+	(+)
,,	,,	,,	2 days	—	+	(+)	—	—
I	Howes	,,	2 days	+	+	+	(+)	—
,,	,,	,,	3 days	—	+	(+)	—	—
II	Pugh	,,	2 days	+	+	+	+	+
,,	,,	,,	2 days	—	+	+	(+)	—
,,	,,	,,	4 days	—	(+)	(+)	—	—

In case of all three cocci the reducing power of the coccus survived its failure to grow in subculture. The results thus confirmed those of the heating experiments. The reducing power, however, did not outlast the death of the coccus for more than a few days.

(*f*) *Effect of Desiccation on the Reducing Power of the Meningococcus.*

This was examined in the same way as when investigating the effect of desiccation on the haemolytic power of the meningococcus. It was found that whereas desiccation has no effect on the haemolytic substance, it completely abolishes the reducing action.

(*g*) *Effect of Menstruum on the Vitality of the Meningococcus.*

When investigating the pathogenicity of the meningococcus it was observed that this micro-organism is more virulent when suspended in broth than when suspended in saline or water ; and the same has

been found to hold with regard to the reducing power of the meningococcus (cf. (*e*) above). The opportunity was now taken of determining the vitality of the coccus when suspended in each of these fluids respectively.

Procedure was as follows: Three equal-sized slope cultures of Type II meningococcus strain Morgan of one day's growth at 37° C. were each suspended in 5 c.c. of distilled water, saline, and broth respectively; 0·5 c.c. of this suspension was then diluted with 4·5 c.c. of the same diluent, and from this a further dilution made in the same way. Thus 0·5 c.c. of the suspensions represented $\frac{1}{10}$ of the slope culture, and the same amount of the dilutions $\frac{1}{100}$ and $\frac{1}{1000}$ of the slope. The test tubes holding the suspensions and dilutions of the coccus in each of the three fluids were placed in a water bath at 37° C., and cultures made from 0·5 c.c. of each of the nine tubes at the start, and after one hour and two hours. The result was as follows:

		Fractions of Slope Culture.		
Menstruum.	*Time.*	1 : 10	1 : 100	1 : 1,000
Water	Start	+	+	—
,,	1 hour	+	(+)	—
,,	2 hours	+	—	—
Saline	Start	+	+	+
,,	1 hour	+	(+)	—
,,	2 hours	+	—	—
Broth	Start	+	+	+
,,	1 hour	+	+	+
,,	2 hours	+	+	+

+ = growth of meningococcus. (+) = slight growth only. — = no growth.

The meningococcus, therefore, perishes more rapidly in water and saline than in broth, and this is the probable explanation of the loss of virulence and of reducing power by the coccus when suspended in saline or water.

(*h*) *Effect of Antiseptics on the Vitality and Reducing Power of the Meningococcus respectively.*

The method used was as follows:

Principle: 0·1 c.c. volumes containing falling amounts of the disinfectant are brought in contact with 0·4 c.c. volumes of a freshly made suspension of the meningococcus in broth containing 10,000 million cocci per c.c.; i. e. with 4,000 million cocci. The test is carried out in small test tubes held in racks in a water bath.

Dilution of Disinfectant. As the disinfectant is diluted 1 : 5 by the coccus suspension, the concentration of disinfectant to add is $\frac{1}{5}$ of the concentration selected for trial. A simple table may be compiled showing how to prepare such amounts rapidly in 0·1 c.c. bulk for testing disinfectants at concentrations between 1 : 10 and 1 : 10,000.

Suspension of the Coccus. A suspension of the meningococcus approximately of the density required can be obtained by suspending a plate culture of it in 20 c.c. of broth, or a slope culture in 2·5 c.c. of broth.

Time and Temperature of Exposure. Thirty minutes in a water bath at 37° C.

Method of Culture. Two large loopfuls of the suspension are transferred to a warm trypagar slope which is placed in the incubator directly it has been inoculated.

The result given by three different disinfectants, namely, mercury bichloride, zinc sulphate, and chloramine T, when tested on two different strains of Type I meningococcus by this method are shown in the following table :

		Howes coccus.		*Smith coccus.*	
Disinfectant.	*Concentration.*	*Vitality.*	*Reduction.*	*Vitality.*	*Reduction.*
Mercury bichloride	1 : 10,000	—	(+)	—	—
	1 : 25,000	—	+	—	—
	1 : 50,000	(+)	+	—	—
	1 : 100,000	+	+	+	+
Zinc sulphate	1 : 100	—	—	—	—
	1 : 200	+	—	—	—
	1 : 300	+	+	+	+
	1 : 400	+	+	+	+
	1 : 500	+	+	+	+
Chloramine T.	1 : 500	—	—	—	—
	1 : 1,000	(+)	+	+	—
	1 : 1,500	+	+	+	+
Control	Broth	+	+	+	+

+ = growth or reduction. (+) = slight growth or reduction.

It is seen that there is a rough correspondence between the vitality of the coccus and its possession of reducing power ; and also between destruction of the coccus by the disinfectant, and its loss of reducing power. Although this correspondence is not always exact—zinc sulphate for instance had more effect on the reducing power than on the vitality of the Howes coccus—it would seem probable from these observations that by determining the smallest amount of disinfectant required to inhibit the reducing power of the meningococcus, some idea can be obtained as to the region of the ' end-point ' of the bactericidal power of the disinfectant for this same micro-organism. Should this relation be substantiated by further experiments, a preliminary examination of the effect of a given disinfectant on the reducing power of the micro-organism—a test easily effected within half an hour or so—may be found to save much time, labour, and material when defining the value of various disinfectants. These remarks apply of course to disinfectants that fail to reduce *per se* in a concentration near their bactericidal end-point.

In addition to the disinfectants mentioned, trial has been made of the effect of other substances such as alcohol, acetone, and ether, also of toluol and phenol. All of these both destroyed the coccus and abolished its reducing power ; but the end-points have not yet been worked out so carefully as in the case of mercury bichloride, zinc sulphate, and chloramine T.

Summary. The reducing agent whereby the living meningococcus when suspended in a suitable medium exercises a strong reducing action on its immediate environment is chiefly confined to the body of the coccus ; in other words to its surface and interior. This

reducing agent survives the death of the coccus brought about by exposing it for 30 minutes to 55° C., but is destroyed by an hour's exposure to 70° C., and practically at once at 100° C. Similarly the reducing agent survives death of the coccus by attenuation. Though not entirely dependent on the vitality of the coccus, the reducing agent does not survive it for long; and the comparatively feeble reducing power of the coccus in water or saline as compared with its power in broth, cerebrospinal fluid, or serum, is associated with the speedy death of the coccus in the former fluids. Again, while the end-points of the three disinfectants tested on the meningococcus corresponded roughly with the concentration at which the reducing power of the coccus was abolished by them, this correspondence was not always exact. Desiccation destroys alike the vitality of the coccus and its intracellular reducing agent.

Conclusion. The inference drawn from these observations is that the reducing action of the living meningococcus is brought about by an intracellular reducing enzyme or 'reductase' contained by this micro-organism.

Note with regard to the Literature of Bacterial Reductase. Reference to the literature shows that the reducing action of bacteria has been known for many years past, and that this reducing capacity is very widely spread among bacteria. There is a difference of opinion as to the manner in which it is brought about; some holding that it is produced by the vital activity of the bacterial cytoplasm, others that it is due to an enzyme secreted by the cell and capable of extending for some distance beyond the actual cell wall. Spina and Rothenberger first showed that the reducing agent was thermolabile, the reaction being destroyed by heating the culture to 60–70° C. Cathcart and Hahn's observations support the view that the reaction is due to an enzyme. In favour of this is the unfavourable influence of low temperatures (0° C.), the optimum results being obtained at 40° C., and an impairment of the reducing agent produced by heating the cultures to 55–60° C. Führmann (*Vorlesungen über Bakterienenzyme*, Jena, 1907, p. 110), to whose monograph I am indebted for the above information, concludes that the balance of the evidence is in favour of the reaction being due to an enzyme which he calls reductase. It will be observed that the experiments related in the present report led to a similar conclusion with regard to the reducing capacity of the meningococcus.

8. Further Observations on the Reductase and Endotoxin of the Meningococcus.

A. Comparison of the Reductase of the Meningococcus with that of *Staphylococcus epidermidis* and of *B. coli* respectively.

(*a*) *Effect of Exposure to a Temperature of* 70° *C.*

A slope culture on trypagar was made of *Staphylococcus epidermidis* and another of *B. Coli*, and after 18 hours at 37° C. the growth on each was suspended in 2·5 c.c. of broth and 1 c.c. of the suspension then heated to 70° C. for one hour. On testing the reducing power of the suspensions before and after they had been heated, it was

found that while before heating both bacteria up to at least $\frac{1}{1\,5\,0}$ of a slope reduced methylene blue, after the heating no reduction was produced even by $\frac{1}{5}$ of a slope. The reductase of both these bacteria, therefore, is no more resistant to a temperature of 70° C. than is that of the meningococcus.

(*b*) *Effect of Desiccation.*

The growths on three one-day slopes of the meningococcus (T. I, Smith) *Staphylococcus epidermidis* and *B. Coli* respectively were each suspended in 2·5 c.c. of broth, and 0·5 c.c. of this suspension was then placed in a watch glass and dried *in vacuo* at 37° C. overnight. Next morning the dry deposit in each watch glass was taken up in 1 c.c. of distilled water, a culture made, and the reducing power tested in the usual way. The result was as follows :

	Fractions of Slope.			
	Reducing Power.			*Vitality.*
	1 : 10	1 : 20	1 : 40	1 : 10
Meningococcus . .	−	−	−	−
Staphylococcus . .	+	+	+	+
B. Coli . . .	+	+	+	+

It appears that whereas after desiccation the meningococcus had lost both its vitality and reducing power, the two hardier bacteria had retained both qualities.

B. Serviceability of the Reductase for detecting the presence of living Meningococci in Body Exudates.

Experiment I. In order to determine if the reductase test can be applied to detect the presence of living bacteria in an exudate an experiment was made in the first place to see if an exudate free of bacteria reduces methylene blue.

A guinea-pig weighing 350 grammes was injected intraperitoneally with 10 c.c. of 10% Witte peptone dissolved in saline, and killed by ether next morning. The peritoneal cavity was found to be filled with a large exudate rich in polymorphonuclear cells, but free of bacteria. By diluting 0·5 c.c. of the exudate through successive 0·5 c.c. amounts of broth a series of dilutions was obtained of 1 : 2, 1 : 4, 1 : 8, 1 : 10, &c., and methylene blue was added to each of these and the tubes kept for two hours at 37° C. Although the leucocytes formed a thick white precipitate in the first tubes no definite reduction of the methylene blue was observed except for a trace in case of 0·5 c.c. of the undiluted exudate. On now adding a rich suspension of living meningococci to the exudate and repeating the test, reduction of the methylene blue was effected up to the seventh successive dilution (1 : 128). It appeared, therefore, that whereas the exudate *per se* failed to reduce when diluted in broth 1 : 2 and more, this exudate did not prevent the meningococci from exercising their reducing power.

Experiment II. The growth on two 1-day-old plates of a virulent Type II meningococcus (Lindenbaum) was suspended in 10 c.c. of equal parts broth and saline and injected intraperitoneally into a guinea-pig of 390 grammes weight. The dose thus given represented

an eight-fold fatal dose. After five hours the guinea-pig was killed by ether and its peritoneal fluid and blood collected and examined for reduction of methylene blue in the usual way. Cultures were also made from measured amounts of the peritoneal exudate and the blood of the guinea-pig. The results were as follows :

Number of living meningococci. The blood of the guinea-pig contained over 100 but less than 1,000 living meningococci per c.c., and the peritoneal exudate over 1,000 but less than 10,000 living cocci per c.c., as judged by cultures made from quantitative dilutions of these materials.

The reducing power of the suspension of cocci injected, of the peritoneal exudate before and after being centrifuged, and of the blood before and after clotting is shown in the following table :

		Dilution in Broth.									
No.	*Material*	*1 : 2*	*1 : 4*	*1 : 8*	*1 : 16*	*1 : 32*	*1 : 64*	*1 : 128*	*1 : 256*	*1 : 512*	*1 : 102*
1	Suspension injected	+	+	+	+	+	+	+	+	+	—
2	Peritoneal exudate	+	+	+	+	+	+	—	—	—	—
3	Do. after centrifugation	+	+	(+)	—	—	—	—	—	—	—
4	Blood before clotting	+	—	—	—	—	—	—	—	—	—
5	Serum	+	+	—	—	—	—	—	—	—	—

+ = reduction of M. Blue.

It would appear that

(1) The suspension was eight times as active as the exudate ; but its dilution by the exudate must be taken into account, also the elapse of five hours since the injection.

(2) The exudate after being centrifuged had lost seven-eighths of its reducing power by removal of a large portion of its particulate matter.

(3) The blood of the same guinea-pig had some slight reducing action on methylene blue better seen when the red corpuscles were removed—possibly because they to some extent neutralized the reducing agent.

Conclusion. The reductase of the meningococcus can be applied to detect the presence of this micro-organism when present in a body exudate in living condition and in large numbers.

C. The Endotoxin of the Meningococcus.

The experimental study of the pathogenic action of the meningococcus described in a preceding section led to the conclusion that two different factors are here involved, namely, (1) a labile element, especially prominent in the most virulent specimens of the meningococcus, and intimately bound up with an ability on the part of the coccus to multiply in the tissues of its host ; and (2) a more stable toxic element, intracellular in origin, and still in evidence when the vitality of the coccus has been destroyed. The object of the present section is to describe the result of further study of this endotoxin of the meningococcus.

This investigation was determined by demands of a very practical nature. During earlier stages of the outbreak an undesirable fluctuation in the therapeutic potency of the antimeningococcus serum then

available was the cause of much concern. This concern was increased when it was found, on comparing in the laboratory the serum that had proved most potent clinically with numerous samples of anti-meningococcus serum of inferior therapeutic value, that none of the three indices of potency chiefly advocated up to that time served to distinguish between them. Thus the superiority of the successful serum lay neither in its opsonin content, nor in its agglutinin content, nor in its yield of complement-fixing antibodies. When, therefore, towards the end of 1917 it was found that of all the specimens of antimeningococcus serum under investigation, the clinically potent serum alone possessed the ability to neutralize the toxic action of the dried meningococcus in the peritoneal cavity of the mouse ; and also, that this toxin-neutralizing capacity of the serum extended to both of the commonest types of the meningococcus, it seemed that the long-sought-for clue to potency had been obtained, and further study of the endotoxin of the meningococcus was indicated. This investigation, however, was prevented for a considerable time by the more pressing need first of testing numerous samples of antimeningococcus serum so as to select the best for immediate clinical use, and secondly by the necessity of obtaining some information as to the conditions under which meningococcus antiendotoxin is to be found in the circulating blood of an animal in course of immunization. Accordingly it was not until the middle of 1918 that an investigation could be begun for the purpose of obtaining further information concerning the endotoxin of the meningococcus.

In the meantime, the method used for determining the antiendotoxic capacity of various samples of serum—though the best that could be elaborated at the time—was far from perfect. The serum that had proved most efficient clinically was found to neutralize 1 M.L.D. of the dried coccus of each of the two commonest types of the meningococcus with 0·5 c.c. of the serum. In testing samples of serum to see if they came up to this standard, it was found that if a dose of powdered dry coccus was administered that exceeded the M.L.D. for the mouse, very few samples indeed of antimeningococcus serum then available were capable of neutralizing it ; and if a sub-minimal lethal dose was used, the controls lost in conclusiveness. It was clear that this dilemma would only be disposed of satisfactorily when in addition to improved knowledge of the conditions under which antiendotoxin is generated—information indispensable for the preparation of more potent serum in quantities sufficient to meet demands—a method could be arrived at of obtaining the endotoxin in a form that would permit of more accurate measurement of its toxic effect. It seemed possible also that a satisfactory solution of the latter problem might even go some way towards resolving the former as well.

For these reasons, the present study of the endotoxin of the meningococcus has been influenced largely by a desire to obtain if possible a method of getting the endotoxin of this micro-organism into solution. In the first place, however, an investigation was made in order to ascertain whether the endotoxin contained by the meningococcus can be increased by raising the virulence of the coccus by animal passage.

(*a*) *Experiments to determine* (1) *if the virulence of the Meningococcus can be increased by animal passage ; and* (2) *if in the event of such increase of virulence, the Endotoxin contained by the Coccus is also increased.*

Four specimens of the meningococcus—two of Type I and two of Type II—were each injected into mice, and on recovery from the heart's blood of the first mouse passed through a succession of further mice in the same way. After several passages, the virulence of each coccus for the mouse was again measured. In only one of these cocci could any increase be detected, namely, in the case of Type I, strain Howes, that before mouse passage killed when alive in a dose of 5,000 millions, not in a dose of 2,500 millions ; but on recovery from the fourth mouse in succession killed in a dose of 500 millions. The virulence of this particular coccus, therefore, had increased tenfold.

In order to determine if this increase of virulence was accompanied by an increase in the endotoxin content of the coccus two procedures were tried. In the first place a portion of the identical suspension of the Howes coccus (that on recovery from the fourth mouse proved fatal in a dose of 500 millions when living) had been dried, and the toxicity of this was compared with that of the same coccus before mouse passage when dried in the same way. No difference, however, could be detected in the toxicity of these two specimens of dried coccus. Secondly, the growth on trypagar plates of the coccus before and after mouse passage was collected, washed in saline, dried, and the toxicity of the watery extracts of the dried cocci, and also the dried cocci themselves compared. Again the toxicity was equal. This experience indicates therefore (1) that since three of the four cocci failed to show increase of virulence, it is exceptional to get an increase in the virulence of the meningococcus by passing it through mice ; (2) that when such increase of virulence does take place, it is not necessarily accompanied by an increase in the portion of the endotoxin of the coccus that survives drying.

(*b*) *Comparison of the value of various Solvents for extracting the Toxin from the dried Meningococcus.*

A comparison was made of the value of distilled water, saline, absolute alcohol, acetone, and ether for this purpose by grinding up 0·1 gramme of the dried coccus (Type I, strain Howes) in an agate mortar and slowly adding 5 c.c. of the solvent. The mixture of each solvent with the dried coccus was then transferred to a centrifuge tube, stood over night in the ice chest and centrifuged. The results were as follows :

(i) Naked-eye appearance of the extracts.

(*a*) Water extract. The fluid is more opaque and the deposit is less abundant than in any of the others.

(*b*) Saline extract. The fluid is slightly opalescent and the deposit is over twice as large as in the case of water.

(*c*) All of the remaining extracts are clear and the cocci completely deposited. The acetone extract was slightly yellow, apparently from some substance extracted from the cocci which deposited in yellow drops when the acetone was evaporated off.

(ii) Microscopic appearance of the deposits.

Films were made of each deposit, fixed, stained, and examined. The results were as follows :

Solvent.	*Deposit.*
Water	Sludge. No formed cocci seen.
Saline	Ditto.
Acetone	Mostly sludge. A few cocci.
Alcohol	Many cocci seen.
Ether	Cocci not disintegrated at all.

(iii) Amount of the dried coccus taken up by each solvent.

The deposits were collected from the centrifuge tubes, dried, and re-weighed. The results were as follows :

	Milligrammes of Dried Coccus.				
Solvent :	Water.	Saline.	Acetone.	Alcohol.	Ether.
Before extraction . . .	100	100	100	100	100
After extraction . . .	14	34	57	55	55

A good deal of the dried coccus was unavoidably lost in pouring the materials from the agate mortar into the centrifuge tubes, and in transferring the deposits in the latter to watch glasses for drying. In spite of this the main result is clear, the water having obviously taken up more than the other solvents.

(iv) Toxicity of the residues.

No material difference could be found in the toxicity of the dried cocci recovered after extraction by the various solvents.

(v) Toxicity of the extracts.

Both the water and saline extracts proved toxic to mice intraperitoneally, the former being the more active. The toxicity of the other extracts was not tested on this occasion ; but a test of similar extracts was made later by evaporating off the solvent, dissolving up the dry deposits in water, and injecting them intraperitoneally into mice. These mice remained unaffected.

Conclusion. Distilled water takes up more of the dried coccus and of its endotoxin than any of the other solvents. In consequence distilled water was adopted in the first place for the routine purpose of extracting the toxin from the dried meningococcus when testing samples of serum for antiendotoxin.

(*c*) *The Value of improved Mechanical Disintegration for extracting Endotoxin from the dried Meningococcus.*

Comparison was now made of the toxicity of extracts prepared with distilled water and (*a*) grinding up the coccus in the usual way, (*b*) adding an equal amount of well-washed and sterilized sand before grinding. Both extracts were lightly centrifuged to throw down the larger particles and the sand before testing them for toxicity. As it was found that the addition of sand increased the amount of toxin in the extract, this procedure was adopted for routine purposes.

At this stage, therefore, the method used for testing sera was to weigh out 0·1 gramme of the dried coccus, to add an equal amount of sand, and after thoroughly grinding up the mixture to slowly add

5 c.c. of distilled water while continuing the grinding. The mixture was then centrifuged lightly in order to throw down the sand. The supernatant fluid which was opalescent in colour was fatal to the mouse intraperitoneally in a dose of 0·1–0·2 c.c. For preserving it, a few drops of ether were added.

(d) The Value of Sodium Hydroxide for getting the Endotoxin of the Meningococcus into Solution.

Numerous experiments were now made in order to determine whether the well-known solvent properties of sodium hydroxide could be made use of to obtain the endotoxin of the meningococcus in solution. In this relation importance attaches to the following points :

(1) The toxicity of NaOH *per se* when administered intraperitoneally to the mouse.
(2) The degree to which it dissolves the meningococcus, and
(3) The extent to which it alters the endotoxin.

(1) Toxicity of NaOH for the mouse.

The M.L.D. of NaOH intraperitoneally for a mouse of 15–20 grammes was found to be 0·5 c.c. of a N/10 solution, or 2 milligrammes of NaOH.

(2) The value of NaOH for dissolving the meningococcus and obtaining its endotoxin in solution.

Experiments with the Dried Coccus. N/1 NaOH was tried in the first place. In a preliminary experiment it was found that by grinding up 0·1 gramme of the dried coccus in 1·25 c.c. N/1 NaOH, by now adding 1·25 c.c. of water, and then placing the fluid in a water bath at 37° C. for one hour, and finally reducing the alkalinity to N/50, a solution of the bacterial protein and endotoxin was obtained with the endotoxin unaltered, as proved by the ability of antiendotoxic serum, but not of other serum to neutralize it. Nevertheless, in further experiments this method proved too uncertain for routine use owing to the tendency of the strong NaOH to destroy the toxin.

An attempt was next made to get the dry coccus into solution by grinding the powder up directly in falling amounts of NaOH, e. g. N/2, N/4, N/10, N/20, &c., subsequently reducing the alkalinity to N/50 where necessary by dilution or neutralization, or by both. As only imperfect solution of the dry coccus took place, however, the method was abandoned.

The effect was now tried of adding falling amounts of NaOH to a suspension of the dried coccus already made by grinding it up in water. As before, the stronger solutions of NaOH were found to destroy the endotoxin, but the weaker solutions (N/20, N/40, &c.) were found to liberate toxin from the particles of dried cocci without destroying it, and to considerably improve the sureness of the killing power of the suspension. For this reason, and also because it saves so much time and labour, the addition of dilute NaOH to the suspension of the dry coccus was henceforth adopted for routine use.

In order to avoid loss of toxicity on keeping, a smaller amount of the dried coccus is used, and the samples of serum tested shortly after

preparing the endotoxin. Procedure is as follows : 0·05 gramme of the dried coccus is ground up in an agate mortar with 1·25 c.c. of distilled water, and after grinding for a few minutes 1·25 c.c. of N/20 NaOH is added. The suspension undergoes rapid clearing when the NaOH is added. This fluid is then transferred to watch glasses already set out for each serum. The dose of dissolved endotoxin used is generally 0·1 c.c. or 0·15 c.c., according to the toxicity of the dried coccus in use ; 0·5 c.c. of serum is then added to each watch glass, normal serum being added to the first pair, and each of the samples of serum for test to the others. The mixtures of serum and toxin are placed in the incubator for 30 minutes, and then the contents of each watch glass are injected intraperitoneally into a mouse. The experiment is carried out in duplicate, so as to reduce error due to the varying susceptibility of individual mice. The mice are kept under observation for 2–3 days and the result recorded. Since this method was elaborated, it has been in routine use for the purpose of testing samples of serum for antiendotoxin.

Experiments with the raw Coccus. Kraus and Doerr observed that the addition of N/10 NaOH to the raw coccus renders the killing power of its endotoxin more sure. This solution, however, is unnecessarily strong, and is certainly too strong when mice are used, since 0·5 c.c. of the N/10 solution *per se* is fatal to them.

Some experiments made with a suspension containing approximately 200,000 raw meningococci per c.c., showed that when present in a concentration of N/40, the NaOH produced partial solution of the cocci and increased the sureness of the killing power of the suspension. This toxicity also was retained by the fluid of the suspension when the cocci were centrifuged out of it. It would appear, therefore, that in case of need endotoxin can be obtained from the raw coccus by this procedure for the purpose of testing the antiendotoxic value of samples of serum. It was observed that the addition of dilute alkali, e.g. N/80 or N/160, to suspensions of the raw meningococcus rendered them very viscid, a change due probably to the liberation of mucin from the coccus.

(*e*) *The Value of Autolysis for the purpose of obtaining a Solution of Meningococcus Endotoxin.*

Some experiments were made in which suspensions of the raw coccus in water or saline were incubated with toluol or ether as preservative in order to ascertain the comparative value of autolysis for the purpose of obtaining a solution of the endotoxin. Although active endotoxin was found free in the fluid in some cases after 24 hours at either 37° C. or laboratory temperature, it was apt to disappear later ; and as on repeating the experiments this method was found to be less sure as a source of active endotoxin than the dried coccus, it was not adopted.

(*f*) *The Effect of Heat on the Endotoxin of the Meningococcus.*

Experiment 1. The effect of heat was tried in the first place on the toxicity of a suspension of Type I meningococcus, strain Smith, prepared by suspending the growth of each of two trypagar plates

of 18 hours' incubation at 37° C. in 5 c.c. of distilled water, and then pooling them. After microscopical examination the suspension was divided into four parts, one of which served as control, while the other three were heated for half an hour to 55° C., 100° C., and 120° C. respectively. The two latter temperatures were obtained by heating the tubes containing the suspension in a water bath of boiling water, and in the autoclave respectively. At the same time also the effect of heat was determined in the same way on the Smith coccus after it had been dried, the suspension used for this purpose being made up by grinding up two lots of 0·1 gramme of the dried coccus in 5 c.c. of distilled water, and then pooling them. Both watery suspensions of the Smith coccus, raw and dried respectively, failed to kill a mouse in a dose below 0·1 c.c. The effect of heat on their toxicity was found to be as follows :

Treatment.	*Dose.*	*Raw Coccus.*	*Dried Coccus.*
Unheated	0·1	+	R
	0·2	+	+
	0·3	+	+
Heated to 55° C. for 30 minutes . .	0·1	+	+
	0·2	+	+
	0·3	+	+
Heated to 100° C. for 30 minutes . .	0·1	+	R
	0·2	+	+
	0·3	+	+
Heated to 120° C. for 30 minutes . .	0·1	+	+
	0·2	R	+
	0·3	+	+

+ = mouse died. R = mouse ill ; recovered.

The mice that succumbed to the raw unheated coccus grew meningococci from their hearts' blood ; the blood of the other mice that died was sterile.

The Smith coccus, therefore, whether raw or dried, withstood heating to temperatures up to 120° C. for 30 minutes without loss of toxicity.

Experiment 2. The experiment was next repeated on Type II, strain Pugh, in the same way. The results were as follows :

Treatment.	*Dose.*	*Raw Coccus.*	*Dried Coccus.*
Unheated	0·1	+	+
	0·2	+	+
	0·3	+	+
Heated to 55° C. for 30 minutes . .	0·1	+	+
	0·2	+	+
	0·3	+	R
Heated to 100° C. for 30 minutes . .	0·1	R	+
	0·2	+	+
	0·3	+	+
Heated to 120° C. for 30 minutes . .	0·1	+	O
	0·2	+	O
	0·3	+	O

+ = mouse died. R = mouse ill ; recovered. O = mouse unaffected.

Whereas the Pugh coccus, when raw, withstood heating to 120°C. without loss of toxicity, the suspension of the same coccus when dried lost its toxicity within half an hour at this temperature.

Experiment 3. The experiment was repeated on two further specimens of Type I and II respectively, strains Howes and Morgan. Suspensions of the raw coccus were used, exposure to 55° C. was omitted, and the effect of exposure to 120° C. for two hours was tried instead. The results were as follows :

Treatment.	*Dose.*	*Howes.*	*Morgan.*
Unheated	0·1	R	+
	0·2	R	R
	0·3	+	+
Heated to 100° C. for 30 minutes . .	0·1	R	+
	0·2	+	R
	0·3	+	R
Heated to 120° C. for 30 minutes . .	0·1	R	O
	0·2	R	O
	0·3	+	O
Heated to 120° C. for 2 hours . .	0·1	R	O
	0·2	O	O
	0·3	O	O

+ = mouse died. R = mouse ill ; recovered. O = mouse unaffected.

It would appear that the Howes coccus retained some of its toxicity after exposure to 120° C. for 30 minutes, but lost it after two hours at this temperature. The toxicity of the Morgan coccus was destroyed within half an hour at 120° C.

Experiment 4. The effect was now determined of heating the raw suspensions used in experiments 1 and 2 to 120° C. for two hours. Sufficient of the suspension of each that had been heated to 55° C. for 30 minutes remained for this purpose. It was found that whereas both these suspensions were toxic to mice before being autoclaved, neither of them showed any evidence of toxicity after being exposed for two hours to 120° C.

Conclusion. These experiments on mice led to the following conclusions. The endotoxin of the meningococcus withstands heating to 100° C. for 30 minutes, and in some cases to 120° C. for this time. At 120° C., however, it is destroyed within two hours. As might, perhaps, have been expected, the loss of toxicity produced by heat is more evident in cocci of low than in those of high toxicity.

Confirmatory Experiment on Guinea-pigs. At a later date, when investigating the question of the secretion of soluble toxin by the meningococcus in the peritoneal cavity of the guinea-pig the opportunity was taken of repeating the above experiment on guinea-pigs. Four large guinea-pigs were selected of between 350 and 450 grammes weight. A recently isolated meningococcus (Type II Lindenbaum) of good virulence was used and eight plates of it of 18 hours' growth suspended in altogether 16 c.c. of equal parts of saline and broth. This suspension was divided into four equal portions, the first of which was used as control, the remainder heated for half an hour to 55° C., 100° C., and 120° C. respectively. Each portion was then injected into the peritoneal cavity of a different guinea-pig. Further

details of this experiment and the results are seen in the following table:

No.	Treatment of Coccus.	Vitality.	Weight of Guinea-pig.	Result.	Peritoneal Fluid. Lesion.	Film.	Culture.
1	Raw	+	380	Dead in 18 hrs.	Haemorrhagic Peritonitis.	Swarming with Cocci.	+
2	Heated for 30 min. to 55° C.	—	450	Do.	Do.	Do.	—
3	Heated for 30 min. to 100° C.	—	390	Do.	Serous Peritonitis.	Do.	—
4	Heated for 30 min. to 120° C.	—	350	Do.	Do.	Do. (Shadows)	—

The dose given to each of these guinea-pigs was about eight times the M.L.D. of the living coccus for a guinea-pig of this size; the dose therefore was a large one. The result, however, goes to confirm the previous observation on mice to the effect that the endotoxin of the meningococcus may withstand heating for 30 minutes to 120° C. It is noteworthy that although the meningococcus in this experiment produced haemorrhagic peritonitis raw, and when heated to 55° C., it failed to do so after being heated to 100° C.

(*g*) *Trial of Vaughan's Method for the purpose of obtaining a Solution of the Endotoxin of the Meningococcus.*

The principle of this method which has been applied by V. C. Vaughan and S. M. Wheeler to *B. Coli* and other bacteria is to boil up the dried bodies of the micro-organism with absolute alcohol containing 2 per cent. of NaOH. By repeated boilings of an hour's duration under a reflux condenser the bacterial protein is split up and the intracellular poison is taken up by the alkaline alcohol, from which it can be recovered by neutralizing the alkali with HCl., and after removing the salt thus formed, evaporating off the alcohol. The deposit left is soluble in water and very toxic.

As this method does not appear to have been applied previously to the meningococcus, an attempt was made to extract the endotoxin from that micro-organism by means of it. The method as described by Wheeler (*Journal of American Medical Association*, April 22, 1908) was followed. A gramme of the dried meningococcus (Type I, strain Smith) was powdered well, extracted with ether in a soxhlet, and again dried. The weight of dried coccus recovered from the soxhlet was 0·8 gramme. This powder was next boiled three times over for one hour in absolute alcohol containing 1 : 50 of NaOH. The alcohol was then neutralized with HCl., filtered, and evaporated to dryness. The yellow scaly deposit thus obtained was dissolved up in water and injected intraperitoneally into mice, but no toxic effect was observed. The residue of the bacterial bodies was now tested in the same way, but again with no result. The prolonged boiling in alkaline alcohol apparently had destroyed the endotoxin of the meningococcus. The antigenic value of the extracted bodies was examined by Major A. S. G. Bell, who found its specific agglutinogenic properties to be intact.

The method was tried once more, but again without success. In this case, however, while the deposit from the alcoholic extract was without toxic action, a little endotoxin was still left in the coccus bodies.

(*h*) *A Method of obtaining the Endotoxin in Solution.*

Although Vaughan's particular method of combining heat with alkali was not successful, nevertheless, in view of the ascertained resistance of the endotoxin of the meningococcus to heat and to dilute alkali separately, it still seemed possible to obtain a solution of it by this means. Accordingly, before the Central C.S.F. Laboratory was demobilized the following experiments were carried out.

Experiment 1. The effect was first tried of boiling the dried coccus in N/40 NaOH (i. e. NaOH 1 : 1,000) dissolved in water. One gramme of the dried meningococcus (Type I, Smith) was put into a flask with 50 c.c. of N/40 NaOH and boiled for one hour in the steamer. The reaction which was now N/110 NaOH was increased to N/40 and the boiling repeated for one hour. After the second boiling the alkalinity which was again N/110 was increased to N/40 and the boiling repeated for one hour. The mixture was now neutralized with HCl. The coccus bodies had largely gone into solution, a clear brown-coloured fluid resulting. This fluid mixed well with water, entering apparently into complete solution with it. On testing the toxicity, however, it was found that 0·5 c.c. of the fluid was required in order to kill a mouse by the intraperitoneal route. As the M.L.D. of the suspension before being heated had been found to be 0·1 c.c., it was evident that the prolonged boiling even in N/40 NaOH had destroyed a large proportion of the endotoxin.

Experiment 2. The experiment was now repeated, but the dried coccus was only boiled for half an hour in the N/40 NaOH, and then neutralized. Although solution was less perfect than in Experiment 1, and the resulting fluid far more opaque, the bacterial protein, nevertheless, was found to keep up well in it on standing. As the M.L.D. for the mouse intraperitoneally was found to lie between 0·1 and 0·2 c.c., but little of the endotoxin had been lost ; and the toxicity was found to be practically the same after the solution had stood for two weeks at laboratory temperature. Since serum containing antiendotoxin for Type I, and neutralizing a suspension of this same Smith coccus in cold water, was also found to neutralize the toxic action of the present solution, the specificity of the endotoxin was unimpaired. While it is not claimed, therefore, that an entirely satisfactory method of obtaining meningococcus endotoxin in solution has yet been obtained, the present procedure would appear to constitute a material advance in that direction.

Summary. Increase in the virulence of the meningococcus resulting from animal passage is not necessarily accompanied by a rise in its endotoxin content. As a means of obtaining meningococcus endotoxin, autolysis is not sure enough to be satisfactory. For extracting endotoxin from the meningococcus, distilled water proved superior to the other solvents tested. The addition of dilute NaOH increases the solvent power of water, and does not destroy the endotoxin.

Meningococcus endotoxin withstands heating for half an hour to 100° C., and sometimes for half an hour to 120° C., but is destroyed within two hours at 120° C. By heating the coccus for not too long a time to 100° C. in dilute alkali its endotoxin becomes distributed through the fluid and this method, or some development of it, promises to be of considerable value in further studies of meningococcus endotoxin.

9. Antibodies to the Meningococcus.

The specific antibodies present in antimeningococcus serum include agglutinin, precipitin, complement-fixing substances, opsonin, lysin, and antiendotoxin.

It has already been related how agglutinin was found to have a direct practical application for the purpose of defining and identifying the meningococcus of one or another type ; thus proving of the greatest use not only in increasing the accuracy of the procedure provisionally adopted for limiting the spread of cerebrospinal fever, but also in enabling a watch to be kept on the general prevalence of the meningococcus in a community when cerebrospinal fever is present, and when it is absent ; and during the rise and fall of an outbreak.

The practical application of bacteriology for the purpose of dealing with cerebrospinal fever, however, is not limited to prevention. Before the recent outbreak began the intrathecal administration of potent antimeningococcus serum had been conclusively shown by Flexner and others to be capable of materially reducing the mortality from this disease. But as yet no sure method of standardizing the serum had been arrived at ; and largely as a result of this defect most of the antimeningococcus serum used clinically during earlier stages of the recent outbreak proved ineffective.

A study, therefore, was undertaken of the antibodies present in antimeningococcus serum, and when a consignment of this serum came to hand that was found clinically to possess a high degree of therapeutic efficacy, the antibody content of this batch of serum was compared at length with that of specimens of less successful serum with a view to defining the cause of its therapeutic superiority. For a considerable time this attempt to discover the antibody chiefly concerned in the therapeutic potency of antimeningococcus serum was in vain. How the desired information eventually came to light, the practical application made of this knowledge, and with what degree of success, will be seen later. The result of the studies that have been made of individual antibodies to the meningococcus will now be described.

10. Agglutinin.

A. *Antigens.*

The chief preparation employed was a saline suspension of the meningococcus prepared in the following manner. The growth from trypagar plates of 18 hours' incubation at 37° C. is suspended in saline and heated to 65° C. for 30 minutes in order to inactivate the autolytic enzyme of the coccus. The density of the suspension is then determined by transferring 0·1 c.c. of it to a clean test tube and

adding water (from a 5 c.c. pipette graduated in tenths of a c.c.) until the suspension is only just perceptibly turbid when compared with a control of water alone. This end-point may be taken to represent a density of 100 million cocci per c.c., and the amount of water needed to dilute the suspension to this point is readily determined in a few minutes. The calculation is a perfectly simple one, e. g. 0·1 c.c. of the suspension requires 10 c.c. of water to reach the end-point; it contains therefore $10 \times 100 = 1{,}000$ million cocci, and a c.c. of the suspension therefore contains 10,000 million cocci per c.c. After a little practice the end-point is easily recognized and the calculation is simplified by realizing that every c.c. of water required by 0·1 c.c. of the suspension in order to reach the end-point indicates the presence of 1,000 million cocci per c.c. of it. The density of the suspension having been determined, it is diluted with saline until it contains 2,000 million cocci per c.c., 0·5 per cent. of phenol is then added and the suspension transferred to a glass-stoppered bottle. Suspensions made in this way keep for several months and can be used either as antigens, or for testing the agglutinating power of sera.

Other antigens tested at one time or another have been the sensitized raw coccus, the dried coccus, the raw coccus after being autoclaved for half an hour at 120° C., and the dried coccus after most of the endotoxin contained by it had been destroyed by boiling it up for three hours in absolute alcohol containing 1 : 50 of NaOH.

B. *Mode of Dosage.*

Rabbits weighing from 1,000 to 1,500 grammes were used, as older rabbits react less specifically. The injections are made intravenously and the rabbits weighed daily. A suitable first dose is 500 million of the raw coccus. Hine's method consists in giving on the first day two doses of 250 millions at an hour's interval, and on the fifth day a dose of 1,000 million. By this procedure the animal's serum may give a clear-cut titre of 1 : 400 to 1 : 800 by the tenth day. Another method is to give the rabbit a dose every 48 hours, beginning with 500 millions and repeating the preceding dose before raising it.

C. *Mode of conducting the Agglutination Test.*

Small glass tubes ½ inch broad by 3 inches long, plugged with wool, are used; they are held in square wooden racks designed by Major Hine which fit into the 55° C. incubator. Each rack holds nine rows of twelve tubes, and a strip of celluloid is nailed between each row and at either end. The principle always followed has been first to dilute the serum in saline, each dilution bulking to 0·5 c.c. When the dilutions have been made, 0·5 c.c. of the saline suspension is run into each tube. An example is as follows:

It is proposed to test a serum at 1 : 50, 100, 200, and 400. Four tubes are put into the rack, the first is left empty, and 0·5 c.c. of saline is run into each of the three others; 0·1 c.c. of the serum is now diluted with 2·4 c.c. of saline, and 0·5 c.c. transferred to each of the first two tubes; after shaking, 0·5 c.c. from the second tube is transferred to the third, and from that 0·5 c.c. to the fourth—0·5 c.c. from the latter being then thrown away. A dilution is thus obtained of 1 : 25, 50, 100, and 200 of the serum; 0·5 c.c. of the suspension is then

added all round the rack placed at 55° C., and the result read off after 24 hours. A control with normal serum in the same way should always be carried out.

D. *Absorption Tests.*

The object of these is to determine whether a given coccus removes the specific agglutinin from a specimen of agglutinating serum in the same manner as the homologous coccus, or not. The titre of the serum for the homologous coccus must be known. A low dilution of the serum is then made and divided into three parts, one of which is saturated by adding to it an equal quantity of the suspension of the homologous coccus, another is saturated in the same way with the test coccus, the third receives an equal amount of saline only. After standing in the incubator at 37° C. over night, the tubes are centrifuged, and the agglutinating titre of the unsaturated serum and of the two saturated sera respectively determined for each of the two cocci. The homologous coccus is found to have removed its own agglutinin. If the test coccus is of the same type, it removes this agglutinin also, but if of another type, the agglutinin is untouched. It is advisable to use freshly made suspensions for absorption tests, and Major Tulloch prefers to use a suspension of 4,000 million cocci per c.c. for saturating the serum rather than a suspension of 2,000 million per c.c.

E. *Agglutinogenic Values of various Preparations of the same Meningococcus.*

It has already been stated that the antigen with which the agglutinating serum was prepared (that was used for identifying the meningococcus during the outbreak) was a saline suspension of the raw coccus heated to 65° C. and then phenolated.

Recently, when comparing the value of various preparations of the same coccus for exciting the production of antiendotoxin, the opportunity was taken of observing also their agglutinogenic capacity. The coccus used was Type I, strain Howes ; and the antigens compared were (1) a suspension of the raw coccus, (2) the same suspension after being sensitized by homologous serum subsequently removed, and (3) a standard suspension of 0·1 gramme of the dried coccus in 5 c.c. of distilled water.

The experiment was performed in duplicate, three rabbits receiving a stationary dose throughout, while in three other rabbits the dose was slowly raised. Further particulars are as follows :

Five doses in all were given of each antigen, the interval between the first two being 72 hours, and between the remaining doses 48 hours. The first dose in case of the raw and sensitized coccus respectively was 500 millions, and in the two rabbits in which the dose was raised the last dose was 700 millions. In case of the dried coccus the first dose was 0·05 c.c. of the standard suspension representing 1 milligramme of the dried coccus, and in case of the rabbit in which the dose was raised the last dose was 0·07 c.c. of the suspension or 1·2 milligrammes of the dried coccus. On the eleventh day of the experiment, and 24 hours after the fifth dose, all six rabbits were bled and their serum tested against the homologous Type I coccus and also against a representative coccus of each of the other three types. All of the six sera

proved negative in a dilution of 1 : 50 against these cocci of heterologous types. The results against the homologous coccus were as follows :

Dilution of Serum.

Antigen.	*Dosage.*	*1 : 100*	*1 : 200*	*1 : 400*	*1 : 800*	*1 : 1,000*	*1 : 2,000*	*1 : 4,000*
Raw coccus :	Stationary	+	+	+	+	+	..	..
	Raised	+	+	+	+	+	+	..
Sensitized coccus :	Stationary	+	..	..	..	..	..	..
	Raised	+	+	+	(+)	..	..	..
Dried coccus :	Stationary	+	+	+	+	+	+	..
	Raised	+	+	+	+	+	+	..

+ = well-marked agglutination.

Inferences drawn from these results were as follows :

1. Since none of the six sera contained agglutinin for heterologous types, all of the antigens were specific.
2. As regards the best antigen, clearly the raw coccus was superior to the sensitized coccus for exciting the production of agglutinin ; though this superiority was reduced when the dose of the sensitized coccus was raised. The two rabbits receiving the dried coccus both gave a full response ; but in their case the dose given was relatively much larger.
3. As regards the effect of raising the dose, it is noteworthy that in case of both raw and sensitized coccus, the rabbit gave a better production of agglutinin with the raised dose. In case of the dried coccus, on the other hand, the stationary dose consisting of 1 milligramme of the dried coccus was sufficient to evoke a maximum response.

F. *The Effect of Autoclaving the Meningococcus on its Antigenic Specificity.*

Shortly before the Central C.S.F. Laboratory was closed Major Tulloch very kindly carried out the following experiment. Saline suspensions of representatives of each of the four types of the meningococcus respectively were autoclaved at 120° C. for half an hour, and a rabbit was then prepared against each. In all cases agglutinin was formed ; but while this agglutinin was active against the ordinary suspension of the raw coccus of the homologous type (heated to 65° C. for 30 minutes and then phenolated) and was strictly specific, it failed to affect the autoclaved suspension which had stimulated its production. The specific agglutinogenic substance of the meningococcus therefore would appear to withstand not only desiccation, but also exposure in the autoclave for half an hour at 120° C. The fact that the specific agglutininogen of the raw coccus withstands heating for 30 minutes to 120° C. was confirmed later by Major Bell who found, however, that this exposure had the effect of reducing it, and that exposure at 120° C. for two hours abolished it altogether. It may be recalled that in an observation previously recorded when applying Vaughan's method of extracting endotoxin to the meningococcus, it was found that the dried bodies of meningococci after being boiled three times over for an hour each time in absolute alcohol containing 1 : 50 NaOH, although their endotoxin had been to a large extent destroyed, still retained their specific agglutinogenic property.

11. Some Observations on Meningococcal Agglutinating Sera and their Production.

By Major A. S. G. Bell, R.A.M.C.

In the following article, in order to avoid tautology, the word serum is used to mean diagnostic agglutinating serum; and by agglutination is meant that process at 55° C. for 24 hours; a phenolated emulsion (2,000 million per c.c. of meningococci, killed at 65° C.) being employed.

To be efficient, a serum must be possessed of four qualities—catholicity, specificity, the property of keeping its agglutinating power well, and fair rapidity in action. The first two of these characteristics are essential; the four will be examined in detail and in sequence.

Catholicity. By catholicity is meant the property a serum possesses of agglutinating all strains of its own type. Agglutinability generally increases with prolonged subculture on egg and trypagar media; recently isolated meningococci may be less susceptible.

Suspensions of Types I and III agglutinate to a more uniform level with their homologous sera than either Types II and IV with their sera.

The annexed tables show this, and also demonstrate a lack of catholicity in the first Type II serum and the IV serum. The first Type II serum (Morgan) almost fails with 30 per cent. of Type II suspensions; the second Type II serum (Lindenbaum) is better, but such a catholic antigen is rare among Type II meningococci at the titre of the sera employed, the agglutinations of which are recorded in these five tables, which was approximately ≠ 1 : 400 with a suspension of homologous antigen.

In the following tables ≠ = complete agglutination; + = well-marked agglutination; and (+) = slight agglutination only.

TABLE A

Type.	*Serum.*	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*Emulsions.*
I.	Littledale	≠	≠	≠	+	(+)	..	..	..	..	Littledale
		≠	≠	≠	+	..	..	..	..	..	Howes
		≠	≠	≠	+	(+)	..	..	..	..	Smith
		+	+	+	(+)	..	..	..	..	..	Sparks
		≠	≠	+	(+)	..	..	..	..	..	Lockton
		≠	≠	≠	+	+	..	..	..	..	Bernstein
		≠	≠	≠	≠	+	..	..	..	..	McCarthy

TABLE B

Type.	*Serum.*	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*Emulsions.*
II.	Morgan	≠	≠	≠	+	..	..	..	..	..	Morgan
		≠	≠	+	..	..	..	..	..	..	Pugh
		+	(+)	..	..	..	..	..	..	..	Lindenbaum
		≠	+	..	..	..	..	..	..	..	Woolman
		(+)	..	..	..	..	..	..	..	..	Gabbitas
		≠	+	+	..	..	..	..	..	..	Brown
		≠	≠	≠	≠	≠	+	(+)	..	..	Bowen
		≠	+	..	..	..	..	..	..	..	Proctor
		≠	≠	≠	+	..	..	..	..	..	Wethell
		≠	≠	≠	+	..	..	..	..	..	Harrison
		+	..	..	..	..	..	..	..	..	Berridge
		+	..	..	..	..	..	..	..	..	Franklin
		≠	+	..	..	..	..	..	..	..	Stansfield
		≠	≠	..	..	..	..	..	..	..	Newman
		..	..	..	..	..	..	..	..	..	Mohawk

TABLE C

Type. *Serum.*	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*3000*	*Emulsions.*
II. Lindenbaum	≠	≠	≠	≠	+	..	..	..	..	..	Morgan
	≠	≠	≠	+	..	..	..	..	..	..	Pugh
	≠	≠	≠	(+)	..	..	..	..	..	..	Lindenbaum
	≠	≠	+	..	..	..	..	..	..	..	Woolman
	≠	≠	..	..	..	..	..	..	..	..	Gabbitas
	≠	≠	≠	≠	+	..	..	..	..	..	Brown
	≠	≠	≠	≠	+	..	..	..	..	..	Bowen
	≠	≠	≠	+	..	..	..	..	..	..	Proctor
	≠	≠	(+)	..	..	..	..	..	..	..	Wethell
	≠	≠	≠	≠	+	..	..	..	..	..	Harrison
	≠	≠	≠	+	..	..	..	..	..	..	Berridge
	≠	+	(+)	..	..	..	..	..	..	..	Franklin
	≠	≠	≠	+	..	..	..	..	..	..	Stansfield
	≠	+	..	..	..	..	..	..	..	..	Newman
	≠	+	+	..	..	..	..	..	..	..	Mohawk

TABLE D

Type. *Serum.*	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*3000*	*Emulsions.*
III. Chatham B	≠	≠	≠	+	+	..	..	..	..	..	Chatham B
	≠	≠	(+)	..	..	..	..	..	..	..	Chase
	≠	≠	+	..	..	..	..	..	..	..	Bunting
	≠	≠	≠	+	+	..	..	..	..	..	Groom
	≠	≠	≠	≠	+	+	..	..	..	..	Wood
	≠	≠	≠	≠	≠	+	+	..	..	..	Morris
	≠	≠	+	..	..	..	..	..	..	..	Loveday
	≠	≠	+	..	..	..	..	..	..	..	Jackson

TABLE E

Type. *Serum.*	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*3000*	*Emulsions.*
IV. Hicks	≠	≠	≠	≠	+	..	..	..	..	..	Hicks
	≠	..	..	..	..	..	..	..	..	..	Wigglesworth
	+	(+)	..	..	..	..	..	..	..	..	Taplow
	≠	≠	(+)	..	..	..	..	..	..	..	R. V.
	≠	≠	≠	..	..	..	..	..	..	..	Myers
	(+)	..	..	..	..	..	..	..	..	..	Jones

The Type I and III sera are satisfactory; one of the Type II's and the Type IV are poor; the second Type II is moderately good. It must be remembered that these agglutinations are done with laboratory specimens; recently isolated meningococci would show less favourable results.

A comparison of B and C indicates the necessity of selecting by experiment the antigen employed for injecting the animal.

More potent sera were evidently required; the titre was raised to ≠ 1 : 1,500 with the Morgan serum (II) and the Hicks serum (IV), and to ≠ 1 : 1,000 with the Lindenbaum serum (II). The results of increasing the titre of the I and III are not shown.

These different titre sera were not made by using dilutions of one serum, but a fresh serum was manufactured from a different animal. This accounts for a lack of mathematical accuracy when B is compared with B (I) and C with C (I), &c.

Type. Serum.	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*3000*	*3500*	*Emulsions.*
II. Morgan	≠	≠	≠	≠	≠	≠	≠	+	+	..	..	Morgan
	≠	≠	≠	≠	≠	+	(+)	..	..	..	..	Pugh
	≠	+	(+)	(+)	..	..	..	..	..	..	..	Lindenbaum
	≠	≠	≠	≠	+	(+)	..	..	..	..	..	Woolman
	+	≠	(+)	(+)	..	..	..	..	..	..	..	Gabbitas
	≠	+	≠	+	+	(+)	(+)	..	..	..	..	Brown
	≠	≠	≠	≠	≠	≠	≠	≠	≠	+	(+)	Bowen
B (I)	≠	≠	≠	+	+	+	(+)	..	..	..	..	Proctor
	+	≠	≠	≠	≠	≠	≠	≠	+	..	..	Wethell
	≠	≠	≠	≠	≠	≠	≠	+	..	..	..	Harrison
	≠	≠	+	(+)	..	..	..	..	..	..	..	Berridge
	≠	+	+	(+)	..	..	..	..	..	..	..	Franklin
	≠	≠	≠	≠	+	+	(+)	..	..	..	..	Stansfield
	+	≠	≠	+	≠	+	..	..	..	..	..	Newman
	+	..	+	(+)	..	..	..	..	..	..	..	Mohawk
II. Lindenbaum	≠	≠	≠	≠	≠	≠	≠	+	..	..	..	Morgan
	≠	≠	≠	≠	≠	≠	+	..	..	..	..	Pugh
	≠	≠	≠	≠	≠	+	..	..	..	..	..	Lindenbaum
	≠	≠	≠	≠	≠	+	..	..	..	..	..	Woolman
	≠	≠	≠	≠	≠	+	(+)	..	..	..	..	Gabbitas
C (I)	≠	≠	≠	≠	≠	≠	≠	+	..	..	..	Brown
	≠	≠	≠	≠	≠	≠	+	..	..	..	..	Bowen
	≠	≠	≠	≠	≠	+	..	..	..	..	..	Proctor
	≠	≠	≠	≠	≠	..	..	..	..	..	..	Wethell
	≠	≠	≠	≠	≠	≠	≠	+	..	..	..	Harrison
	≠	≠	≠	≠	≠	..	..	..	..	..	..	Berridge
	≠	≠	≠	+	..	..	..	..	..	..	..	Franklin
	≠	≠	≠	≠	≠	+	..	..	..	..	..	Stansfield
	≠	≠	≠	+	..	..	..	..	..	..	..	Newman
	≠	≠	≠	≠	..	..	..	..	..	..	..	Mohawk

Type. Serum.	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*3000*	*Emulsions.*
IV. Hicks	≠	≠	≠	≠	≠	≠	≠	+	+	(+)	Hicks
	≠	+	..	..	..	..	..	..	..	..	Wigglesworth
	≠	≠	+	(+)	..	..	..	..	..	..	Taplow
	≠	≠	≠	≠	≠	+	+	..	..	..	R. V.
E (I)	≠	≠	≠	≠	≠	+	..	..	..	..	Myers
	+	+	(+)	..	..	..	..	..	..	..	Jones

Annexed is a list of the agglutinations of emulsions made from primary cultures of meningococci isolated from the C.S. fluid.

The sera all had a titre ranging between ≠ 1 : 1,000 to ≠ 1 : 1,200. It will be seen that with a single exception, the highest complete agglutination was not above 1 : 400, and several of the Type II cocci did not give complete agglutination above 1 : 100.

These fourteen agglutinations are not selected, but were the last fourteen agglutinations done from primary cultures in one of the laboratories of the Central C.S.F. Laboratory.

Occasionally a recently isolated meningococcus is hypersusceptible to agglutination, and after 24 hours will show complete agglutination with all four types in dilution up to 1 : 400. To avoid this eventuality the rack containing the agglutination of a coccus isolated from the C.S. fluid should always be examined at four and eight hours after it is put in the incubator if possible.

	Type I Serum.				*Type II.*				*Type III.*				*Type IV.*			
Coccus.	*100*	*200*	*400*	*800*	*100*	*200*	*400*	*800*	*100*	*200*	*400*	*800*	*100*	*200*	*400*	*800*
Pay	≠	≠	+	..	(+)	..	..	..	(+)	..	..	..	..	..	..	..
Huddy	≠	≠	(+)	..	(+)	..	..	..	..	..	..	..	(+)	..	..	..
Aldred	≠	≠	+	..	..	..	..	..	..	..	..	..	+	..	..	..
Mellor	≠	≠	+	..	..	..	..	..	+	..	..	..	(+)	..	..	..
Quaterman	..	..	..	..	+	(+)	..	..	..	..	..	..	(+)	..	..	..
Smith	..	..	..	..	≠	+	(+)	..	..	..	..	..	(+)	(+)	..	..
Shaw	..	..	..	..	≠	≠	+	..	(+)	..	..	..	..	..	..	..
Greenway	..	..	..	..	≠	≠	≠	+	..	..	..	..	(+)	..	..	..
McInverly	..	..	..	..	≠	≠	+	(+)	..	..	..	..	..	..	..	..
Monagh	+	(+)	..	..	+	+	..	..	(+)	..	..	..	..	..	..	..
McKay	..	..	..	..	≠	≠	+	..	(+)	..	..	..	..	..	..	..
712	(+)	(+)	..	..	≠	+	..	..	(+)	(+)	..	..	..	..	..	..
890	..	..	..	..	≠	≠	≠	+	..	..	..	..	..	..	..	..
Prayne	+	..	..	..	+	..	..	..	≠	≠	≠	≠	+	..	..	..

These tables, then, demonstrate two things, first, the advisability of a titre of at least 1 : 1,000, and secondly, the selection of a catholic antigen.

In the experiments in search of the latter, great difference will be found in the agglutinogenic properties of different meningococci. Each recently isolated meningococcus (especially of Type II) should be injected into a rabbit and the resulting serum tested.

By this means alone can one hope to be possessed of sera which embrace the majority of the prevailing strains in sporadic cases.

Specificity. The difficulty that at once arises with the employment of a serum with a titre over 1 : 400 is the presence of group agglutinins for the heterologous types, which in the lower dilutions confuse the diagnosis of type. These remarks apply to III in a greater degree than to the other types.

This difficulty can be overcome by finding the end-point of each coccus with the four type sera. Agglutination is present in much higher dilutions with the homologous than the heterologous sera. This method, while practical with a single organism isolated from the C.S. fluid, is quite out of the question when dealing with a large number of cultures resulting from nasopharyngeal swabbing of a number of contacts.

A process of absorption is employed; the group agglutinins are removed from the more potent monotypical sera by saturating them with cocci of the heterologous types.

Technique. From 25,000 million to 150,000 million cocci of each heterologous type killed by heating to 65° C. are added per c.c. of the serum under treatment. The higher amount of coccus is used when the serum produces marked agglutination at 1 : 100 with the heterologous type. Details as follows :

Grow a large number of plates, wash off in a few c.c. of saline. Kill at 65° C., phenolate, centrifuge in four tubes for 2 hours, decant the supernatant fluid, add the serum to one tube, stir up, put in a bottle, shake well, incubate at 37° C. for half an hour, pour on to the cocci in the second tube, incubate for half an hour, pour on the third tube, and so on. The total incubation need not be more than 2½ hours. Shake the bottle well four times during this period. Respin till the serum is clear, decant and test. This process, so far as is known, does not interfere with the keeping power of the serum. Samples

so treated have not lost more titre than their controls in five months.

By this absorption the original titre of a serum may be lowered about 30 per cent. ; it is therefore well to start with a titre of 1 : 1,500. Type III loses more than the others. This saturation method is tedious but effective. The tables below show a serum before and after saturation. The following amounts per c.c. of serum were used :

100,000 of Type I
,, ,, ,, II
,, ,, ,, III.

Before Saturation.

Type.	*Serum.*	*100*	*200*	*400*	*600*	*800*	*1000*	*1500*	*2000*	*2500*	*Emulsion.*
IV.	Hicks	+	+	≠	≠	≠	≠	≠	≠	..	Hicks
		≠	+	(+)	..	..	..	..	..	..	Littledale (I)
		≠	+	..	..	..	..	..	..	..	Morgan (II)
		≠	+	+	..	..	..	..	..	..	Chatham B (III)

After Saturation.

+	+	≠	≠	≠	≠	≠	+	..	Hicks
..	..	..	..	..	..	..	..	..	Littledale (I)
(+)	..	..	..	..	..	..	..	..	Morgan (II)
+	..	..	..	..	..	..	..	..	Chatham B (III)

These absorbed sera should be tested against a number of homologous cocci, and the average titre obtained reckoned as the titre of the serum : not the titre which results from agglutination with an emulsion of its own antigen.

Retention of titre. Regarding the powers of these sera of keeping their titre, low-titred sera cannot be guaranteed to keep it for more than four months. This especially applies to Type II. Higher titred sera apparently keep their titre better ; there is a serum in this laboratory which has kept its titre of ≠1 : 3,200 unimpaired for 20 months ; it has been most of this time stored at room temperature in a dark cupboard ; out of nine sera with an average titre of ≠1 : 2,500 only one had lost its titre when examined at an interval of 9 months.

An effort was once made to dry these sera with the expectation that in this state they would retain their agglutinating properties indefinitely. The three following methods were tried :

(*a*) Large dishes containing a shallow layer of serum (with a small quantity of ether added) in a 37° C. incubator.

(*b*) Desiccation over H_2SO_4 with the air exhausted at 37° C.

(*c*) The passage of a current of artificially dried air over the serum.

On grinding up the brown scaly matter remaining with a quantity of distilled water sufficient to restore to the original bulk, the sera were found to have lost 20–30 per cent. of their original titre.

This was a small matter, as a higher titre could be employed to start with.

But these dried sera all gradually lost their potency, and, some three months later, of the dozen and a half samples not one had a titre greater than ≠ 1 : 100 when ground up with the correct quantity of distilled water.

The reason remained obscure ; the samples were kept in glass-stoppered bottles at room temperature in a dark cupboard.

Rapidity of agglutination. Sera with a titre of ≠ 1 : 400 generally show complete agglutination in 18 to 24 hours. Results when a serum with a titre of ≠ 1 : 1,000 is used can generally be read in about 6 hours. The time varies with different meningococci, but the above times are a fair average.

The real difficulty in the whole matter is to get rabbits to yield a serum with a sufficiently high titre. Various methods of injection were employed, none entirely satisfactory, but the one about to be described gave the best results.

The antigen used is a suspension of a 24 hours' growth of the coccus on trypagar heated to 65° C. for 30 minutes, and phenolated. The injections are given intravenously.

The first dose is a 100 millions of the coccus per 100 grm. of rabbit weight ; weigh the animal daily at the same hour (on account of meals), and be guided entirely by its weight whether it receives a similar dose or no. If its weight be below the original, withhold the dose until it recover ; if its weight be above or equal to the original, repeat the dose.

At the commencement of the second week the dose should be doubled, but again let its administration or the reverse on any day be indicated by the weight.

At the commencement of the third week the dose should be one-eighth slope of the live coccus ; from thence onwards, live coccus in this dose should be given whenever the original weight is shown.

Needless to say, different cocci—even of the same type—vary greatly in toxicity, and some variation of the above unit may be necessary ; only experiment can tell which are the more poisonous strains, but the above is a fairly safe dose to give as a routine, and death following this method of injection is rare. Rabbits will sometimes get into a marasmic condition after several doses, and start losing weight, and although further injections be withheld, will gradually grow thinner and die.

Too much emphasis cannot be laid on watching the weight.

Repeated examinations have shown that an animal losing weight is not making antibodies for the meningococcus, or, at any rate, not agglutinins. Animals treated as described above may show a titre of ≠ 1 : 1,500 on the tenth day, but three weeks is much more commonly required.

The following observations were made from 350 rabbits injected for the production of agglutinating sera.

The main factor influencing the production of agglutinins is the idiosyncrasy of the animal. One animal will give a satisfactory yield of agglutinins, whilst another of identical weight and age and under similar treatment remains refractory. It was found that young vigorous bucks of about 1,500 grm. gave the most satisfactory results. It was thought that the Dutch strain was somewhat better than the others.

Next in importance to the idiosyncrasy of the animal is the antigen employed ; different strains vary considerably in their agglutinogenic properties. Experiment can alone determine the best.

The animals must be kept healthy. It is therefore desirable to keep them in pens (numbered bands of soft aluminium attached to the

hind leg seldom come off). These should be upturned boxes with a hole in the side for the animals to sleep in. If the animals must be kept in cages these should be covered with sacking at night. Warmth is necessary to well-being. As regards food, a liberal diet is required, including one large cabbage leaf per day for each animal. Rabbits deprived entirely of green stuff almost immediately start losing weight ; on the other hand, a diet almost entirely of green food, such as is sometimes given in spring when it is plentiful, interferes with the yield of agglutinins. Groups of animals were fed on diets in which carbohydrates, hydrocarbons, and greenstuffs predominated, and a fourth in which all food was moistened with broth to increase the nitrogenous content. The broth tended after a week to scour the animals, otherwise the animals ate the diets well, but the evidence was too conflicting to allow of tabulation. These remarks on the health of the rabbit may appear trivial, but with an antibody difficult to obtain, attention to these details is necessary, and by watching the weight carefully over-immunization is avoided.

A great help in increasing the yield of the serum is to bleed any animal that gives a satisfactory titre intermittently. By this means the serum obtained from an animal can be doubled or more.

The proximal margin of the ear—the one not generally used for injection—is shaved, and the ear warmed over a carbon filament lamp. The large vein situated there is punctured with a splinter of glass made from a broken microscope slide. It is quite easy to take 20 c.c. of blood in 5 minutes from a 1,500 grm. animal. 100 c.c. have been taken from six animals in 30 minutes by the clock. It is important for the assistant holding the rabbit not to grip it as if in a vice ; this causes the veins to be constricted.

After bleeding, the animal is kept in a box warmed with a carbon-filament lamp, and given a bran mash. The process is repeated on the two following days, and the animal killed on the fourth day.

The serum is tested daily to see that it is not losing its titre, but if the original titre be high, say $\neq 1 : 2{,}000$, it rarely does this.

The serum from the final bleed is milky, but apparently this does not interfere with its agglutinating properties.

Animals so treated often do not lose weight, and remain lively and well in spite of the loss of blood.

For identification of the meningococcus by agglutination the experience of this laboratory points to the advisability of using sera with an average titre of $\neq 1 : 1{,}000$, these sera having been rendered specific by saturation with the three heterologous types.

12. On a rapid method of determining the Serological Type of a Meningococcus by the Agglutination Test.

By Major A. S. G. Bell, R.A.M.C.

Clinical experience shows the value of early administration of monotypical curative serum (see *Report to the Medical Research Committee* on ' The Specific Treatment of Cerebrospinal Fever, with an analysis of the Reports on the first ninety cases treated with Monotypical Sera '). The possibility of injecting suitable monotypical serum at

an early stage of the attack obviously depends on early typing of the infecting meningococcus.

If the standard method of agglutination at 55° C. be employed, 72 hours generally elapse between the lumbar puncture and the type being determined.

With the rapid method herein described, 20–24 hours only are needed to type ; homologous curative serum can then be given.

Technique. Use a pipette made from glass tubing, drawn to a *very* fine point and fitted with a teat. On a glass plate drop 1 drop of each of the four type sera ; alongside each of these drop an equal volume of the emulsion of the coccus under examination ; this should be 40,000 millions to 50,000 millions per c.c. Mix and examine the four pools with a watchmaker's glass. Type is indicated by the pool in which agglutination *first* appears.

After 5 minutes if a negative result obtain, arrange the serum on the plate thus :

Type I.	*Type II.*	*Type III.*	*Type IV.*
2 drops	2 drops	2 drops	2 drops
3 ,,	3 ,,	3 ,,	3 ,,
4 ,,	4 ,,	4 ,,	4 ,,

To each of these pools of serum add a drop of emulsion, mix and examine as before.

If the result is not specific, i. e. agglutination occurs in two pools within two minutes of each other, vary the *emulsion* thus :

3 drops	3 drops	3 drops	3 drops
6 ,,	6 ,,	6 ,,	6 ,,
12 ,,	12 ,,	12 ,,	12 ,,

To each of these add one drop of Type I serum in the first vertical row, one drop of Type II in the second row, and so on. Mix and examine as before. Agglutination will no longer appear with the heterologous serum. If there be agglutination with both I and III and a specific reading cannot be obtained, the type may be called provisionally a I, as the Type I serum, so far as is known, is particularly specific.

If there be not sufficient growth to allow of a 40,000 million emulsion, take a few drops of saline, pick off colonies and make an emulsion. Now instead of a pipette use a platinum loop to make the several mixtures.

General Rules. 1. If the coccus has been grown on blood, get rid of as much as possible of this before making the emulsion.

2. Always kill the coccus at 65° C. before testing.

3. In the end agglutination will generally appear with all four type sera and even with the normal serum. This agglutination may be disregarded. Again, if by varying the emulsion specificity cannot be obtained, and at least one minute does not elapse between the agglutination by two sera of different types, the result should be disregarded and the patient treated with pooled serum till the type can be determined by the usual slow method.

4. Always confirm results by the slow method at 55° C.

Results of Laboratory Tests.

With 15 laboratory specimens of Type I :

Correct answers.	*Negative.*	*Incorrect.*
13	1	1

With 25 laboratory specimens of Type II :

Correct answers.	*Negative.*	*Incorrect.*
24	1	0

With 12 laboratory specimens of Type III :

Correct answers.	*Negative.*	*Incorrect.*
12	0	0

With 7 laboratory specimens of Type IV :

Correct answers.	*Negative.*	*Incorrect.*
4	3	0

As Type IV is so relatively rare this is of small moment.

With fourteen recent cases of C.S.F. a correct answer, as subsequently confirmed by the slow method, has been given in twelve ; one was negative and one wrong.

All these results were obtained in under 24 hours after lumbar puncture.

This method is at present only used in emergency for typing cocci obtained from post-nasal swabs, as agglutination has been known to take place with Gram-negative nasopharyngeal cocci which do not absorb and therefore are probably not true meningococci.

Too much weight must not be laid on the results obtained with laboratory specimens, as experience has shown that meningococci under prolonged culture on egg and trypagar media may tend to become more specialized than when recently isolated.

The general correctness of the results obtained with actual cases indicates that this method should be tried in view of the simplicity of the technique. Any medical man with sufficient skill to add blood aseptically to a slope, and possessed of a 37° C. incubator can grow the meningococcus ; the putting up of dilutions of varying strength and the need of a 55° C. incubator are dispensed with.

The method of manufacture of these high titre sera has been described. A titre of 1 : 2,000 is desirable to start with.

It is necessary to get rid of the group agglutinins which are always present with sera of this strength. These group agglutinins are eliminated by saturating them with cocci of the heterologous types in the manner described in the preceding section.

13. Complement-fixing Antibodies to the Meningococcus.

By Major A. S. G. Bell, R.A.M.C.

The relative abundance of complement-fixing antibodies to individual types of the meningococcus has been determined in case of the following :

i. Therapeutic Sera ;
ii. Agglutinating Sera ;
iii. Serum from cases of Cerebrospinal fever ;
iv. Serum from cases infected by Micro-organisms other than the Meningococcus ;
v. Serum from chronic carriers of the Meningococcus.

Technique. Two different antigens have been made use of so far. At first suspensions of the stock cocci were used in a strength of 200 million organisms per c.c., of known serological type ; this strength being chosen because an emulsion of 400 million per c.c. was found to be slightly anticomplementary. Later, at the suggestion of Captain Thomson, of the Military Hospital, Rochester Row, who has been studying the test in relation to gonococcal infection, an antigen was made by dissolving the cocci in N/20 NaOH and bringing the solution back almost to neutral point by the addition of N/20 HCl. This antigen is used in a dilution equivalent to 100 million cocci per c.c. It is found to be a much more sensitive antigen than the stock suspension, though less specific with a serum rich in antibodies.

The patient's serum was tested in dilutions of 1 : 50, 1 : 100, and 1 : 200. It was found to be advisable to use all three dilutions for each test, as the readings are not sufficiently clean-cut for a single dilution only to be used. Therapeutic or agglutinating sera were tested in still higher dilutions. A sheep haemolytic system was used and complement was obtained from the ear of a guinea-pig.

In the titration of the complement the following method was followed :

Increasing doses of a 1 : 10 solution of complement, viz. 0·15 c.c., 0·2 c.c., and 0·25 c.c., &c., were added to tubes already containing antigen and 1 : 50 normal serum in the proportions subsequently used in the actual test. These were incubated in the 37° C. water bath for half an hour before the haemolytic couple was added. The exact amount of complement required to bring about complete haemolysis under these conditions was then used in the test proper. A control was put up of the serum under observation without the antigen, and a further control of the antigens only.

(i) *Comparison of the Results given by Monotypical Therapeutic sera with the Complement Fixation and Agglutination Tests respectively.*

In the complement-fixation observations dissolved antigens as described above were used. The agglutinating capacity of the sera

TYPE I. THERAPEUTIC SERUM (M.R.C.)

Serum.	1						2						3						4						Test.
	1,000	2,000	4,000	8,000	16,000	32,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	
26 a	≠	≠	≠	≠	+	(+)	≠	≠	+	(+)	..	..	≠	≠	≠	+	..	..	+	+	..	..	..	..	Complement fixation
	≠	+	..	..	..	..	(+)	(+)	..	..	..	..	+	+	..	..	..	..	≠	+	..	..	..	..	Agglutination
6	≠	≠	≠	+	(+)	..	≠	(+)	..	..	..	..	≠	≠	≠	≠	+	..	≠	≠	+	..	..	..	Complement fixation
	≠	≠	≠	..	..	..	..	..	..	..	..	..	≠	≠	..	..	..	..	≠	+	(+)	..	..	..	Agglutination
3	≠	≠	≠	≠	+	..	≠	+	(+)	..	..	..	≠	≠	≠	≠	≠	+	≠	≠	≠	+	..	..	Complement fixation
	≠	≠	≠	(+)	..	..	(+)	..	..	..	..	..	≠	≠	+	..	..	..	≠	(+)	..	..	..	..	Agglutination
31	≠	≠	≠	+	+	..	≠	(+)	..	..	..	..	≠	≠	≠	+	+	..	≠	≠	+	+	..	..	Complement fixation
	≠	≠	+	..	..	..	≠	+	..	..	..	..	≠	+	..	..	..	..	≠	≠	(+)	..	..	..	Agglutination
22	≠	≠	≠	+	(+)	..	(+)	..	..	..	..	..	≠	≠	≠	+	(+)	..	≠	(+)	(+)	..	..	..	Complement fixation
	(+)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Agglutination

E 2

TYPE II. THERAPEUTIC SERUM (M.R.C.)

Serum.	2						1						3						4						Test.
	1,000	2,000	4,000	8,000	16,000	32,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	
15	≠	≠	≠	+	(+)	..	+	(+)	..	..	..	..	+	+	..	..	..	..	(+)	..	..	..	..	..	Complement fixation
	≠	+	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Agglutination
17	≠	≠	+	+	(+)	(+)	≠	≠	+	..	..	..	≠	≠	(+)	..	..	..	≠	(+)	..	..	..	..	Complement fixation
	≠	≠	..	..	..	..	≠	(+)	..	..	..	..	+	+	(+)	..	..	..	≠	≠	..	..	..	..	Agglutination
33	≠	≠	+	+	..	..	≠	≠	≠	+	..	..	≠	≠	≠	≠	≠	+	≠	≠	≠	≠	+	..	Complement fixation
	≠	+	..	..	..	..	+	(+)	..	..	..	..	≠	≠	≠	+	..	..	≠	≠	≠	..	..	..	Agglutination

67

TYPE III. THERAPEUTIC SERUM (M.R.C.)

Serum.	3						1						2						4						Test.
	1,000	2,000	4,000	8,000	16,000	32,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	
X	≠	≠	≠	≠	≠	..	≠	≠	+	(+)	(+)	..	≠	(+)	(+)	..	..	..	(+)	(+)	..	..	..	..	Complement fixation
	≠	≠	≠	+	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Agglutination
21	≠	≠	≠	+	..	..	+	+	(+)	(+)	..	..	≠	..	..	..	..	..	(+)	..	..	..	..	..	Complement fixation
	≠	≠	≠	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Agglutination

TYPE IV. THERAPEUTIC SERUM (M.R.C.)

Serum.	4						1						2						3						Test.
	1,000	2,000	4,000	8,000	16,000	32,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	
43	≠	≠	≠	+	..	..	≠	≠	≠	(+)	..	..	≠	≠	≠	≠	+	..	≠	≠	+	(+)	..	..	Complement fixation
	≠	≠	+	..	..	..	+	≠	≠	≠	..	..	≠	≠	≠	..	..	..	≠	+	(+)	..	..	..	Agglutination

was tested against suspensions of the meningococcus in the standard manner described in the section on agglutinin.

The sera here examined were derived from horses which had been prepared by Dr. Stanley Griffith; each horse had been prepared against a single serological type of the meningococcus, but with a plurality of strains of that type. The antigen used for the horses was the raw coccus, and all of the injections were made intravenously.

In the first series of columns in the foregoing tables the results with the homologous antigen are shown, and in the remaining columns the results with antigens of heterologous types of the meningococcus.

To avoid unnecessary figures the homologous columns start with dilutions of serum of 1 : 1,000, the heterologous with dilutions of 1 : 125.

The sign $\neq$ = complete fixation of complement or agglutination.

The sign + = well-marked fixation of complement or agglutination.

The sign (+) = slight fixation of complement or agglutination.

From the results recorded in these tables it will be seen that as was to be expected, complement fixation is a more delicate test than agglutination.

It may be mentioned that in no less than 28 out of 35 comparative tests between these two reactions, complement fixation gave positive results at a higher dilution than agglutination. In the remaining 7 there was a positive reaction with both methods at the same dilution, and in four of these the agglutination was well marked, whereas the complement fixation was feeble.

There is apparently no fixed relationship between these two processes; for instance, with complement fixation one serum gave a positive reaction at a dilution of 1 : 16,000, while agglutination ceased after 1 : 1,000; and with a second serum the end-point with agglutination was 1 : 8,000, and with complement fixation 1 : 16,000.

It will be observed that as a rule the complement-fixing substances were far more plentiful for the homologous type of coccus than for heterologous types. The result of the complement-fixing reaction thus confirms that of the agglutination test with respect to the serological specificity of the different types of the meningococcus.

(ii) *Monotypical Agglutinating Serum. Comparison of Complement fixation and Agglutination Results.*

The agglutinating sera examined were specimens of the monotypical agglutinating sera prepared in the Central C.S.F. Laboratory and sent out to district laboratories for identification of the meningococcus.

These sera were prepared from young rabbits, each of which had been injected intravenously with a single strain of the meningococcus.

The results are shown in the tables on p. 70. As before, the readings with the homologous antigen are recorded in the first columns.

Again, complement fixation is seen to be more delicate than agglutination. The difference between the end-points of the two reactions, however, is less than in case of the therapeutic sera.

TYPE I. AGGLUTINATING SERUM

Serum.	1						2						3						4						Test.
	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	125	250	500	1,000	2,000	4,000	
Type I. F.	≠	≠	≠	≠	≠	..	≠	≠	≠	+	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Complement fixation
	≠	≠	≠	+	..	..	≠	+	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Agglutination
Type I. G.	≠	≠	≠	≠	(+)	..	≠	+	..	..	..	..	+	(+)	..	..	..	..	..	..	..	..	..	..	Complement fixation
	≠	≠	≠	+	..	..	+	..	..	..	..	..	+	..	..	..	..	..	..	..	..	..	..	..	Agglutination
Type I. J.	≠	≠	≠	≠	(+)	..	+	..	..	..	..	..	≠	+	(+)	..	..	..	(+)	(+)	..	..	..	..	Complement fixation
	≠	≠	≠	≠	..	..	(+)	..	..	..	..	..	+	(+)	..	..	..	..	..	..	..	..	..	..	Agglutination

TYPE II. AGGLUTINATING SERUM

Serum.	2						1				3				4				Test.
	125	250	500	1,000	2,000	4,000	125	250	500	1,000	125	250	500	1,000	125	250	500	1,000	
Type II. E.	≠	≠	≠	≠	+	..	(+)	..	..	..	(+)	(+)	..	..	≠	≠	..	..	Complement fixation
	≠	≠	≠	+	..	..	(+)	..	..	..	+	(+)	..	..	≠	+	..	..	Agglutination
Type II. J.	≠	≠	≠	+	(+)	..	≠	(+)	..	..	≠	+	..	..	+	(+)	..	..	Complement fixation
	≠	≠	≠	+	..	..	+	..	..	..	..	..	..	..	..	..	..	..	Agglutination

TYPE III. AGGLUTINATING SERUM

Serum.	3						1				2				4				Test.
	125	250	500	1,000	2,000	4,000	125	250	500	1,000	125	250	500	1,000	125	250	500	1,000	
Type III. K.	≠	≠	≠	≠	≠	+	≠	≠	≠	(+)	≠	(+)	..	..	..	..	..	..	Complement fixation
	≠	≠	≠	≠	(+)	..	≠	(+)	..	..	(+)	..	..	..	..	..	..	..	Agglutination
Type III. N.	≠	≠	≠	≠	..	..	≠	+	(+)	..	≠	+	..	..	+	(+)	..	..	Complement fixation
	≠	≠	≠	+	..	..	≠	+	..	..	+	+	..	..	..	..	..	..	Agglutination

The specificity of the complement-fixing antibodies for their homologue is seen to be more sharply marked in these agglutinating sera than in the therapeutic sera. This was to be expected, since the rabbits had not been under immunization for as long a time as the horses ; and each rabbit had only been prepared against a single strain, instead of a plurality as in case of the horses.

(iii) *Serum from Cases of Cerebrospinal Fever.*

The complement-fixing power of serum from cases of cerebrospinal fever was tested in a concentration of 1 : 50, 1 : 100, and 1 : 200 in the presence of each of the four types of meningococcus separately.

The stage of the disease at which the serum was examined, the type of meningococcus infecting the patient, and the degree to which the patients' serum reacted in the presence of each of the four types of meningococcus respectively, are set forth in the table on p. 72.

In these 27 cases it will be seen that with one exception (B) the results are not clean cut.

All four type antigens when in the presence of dilutions of the patient's serum fix complement, though not to an equal extent.

An examination of the table will show that in the majority of cases complement is fixed more completely, or in higher dilutions, in the presence of an antigen of the same type as the infecting coccus.

Marked fixation of complement was found in no case prior to the fourth day of disease ; but the exact time after the first onset at which antibodies are present in sufficient quantities to do this is doubtful.

The complement fixation test might be used for the quick determination of type. A considerable proportion of negative results would occur, but the table suggests that the positive ones would generally indicate correctly the type of infecting coccus.

The whole matter is complicated by the fact that in most cases curative serum has been injected intrathecally before a sample of the patient's serum can be obtained.

Some of the antibodies in the patient's serum may be from this source, though when the fluid content of the body is considered the dilution of the curative serum is evidently great. Further case (B), which is clean cut, had only received pooled 1 and 2 therapeutic serum, and J and O had received no intrathecal injection when the patient was bled.

In the controls which were carried out with the serum from men, so far as was known free of any infection whatever, there was no fixation of complement in the presence of the four type antigens. There was one exception to this ; the serum of one of the workers in this laboratory was never used in the titration of complement as it was slightly anti-complementary in presence of meningococcus antigen ; a nasopharyngeal swab gave a pure plate of Type II meningococcus.

No. of Case.	*Day of disease.*	1			2			3			4			*Serum control.*	*Form of antigen.*	*Type of meningococcus infecting patient.*
		1 : 50	*1 : 100*	*1 : 200*	*1 : 50*	*1 : 100*	*1 : 200*	*1 : 50*	*1 : 100*	*1 : 200*	*1 : 50*	*1 : 100*	*1 : 200*			
A	60	≠	..	..	≠	≠	..	+	+	..	+	..	..	..	E	II
B	14	..	..	..	..	..	..	≠	+	..	..	..	..	..	E	III
C	5	≠	≠	≠	..	..	..	≠	≠	(+)	..	..	..	..	E	I
D	? 27	≠	≠	+	≠	≠	(+)	≠	≠	(+)	≠	+	+	..	E	I
„	? 41	≠	+	+	≠	+	..	≠	≠	+	+	..	..	..	Da	I
E	5	+	..	..	≠	+	..	≠	≠	..	≠	..	..	..	E	III
„	9	≠	+	+	≠	≠	+	≠	≠	≠	≠	+	+	..	Da	III
F	30	+	..	..	≠	+	..	(+)	..	..	(+)	..	..	..	E	II
G	35	..	+	..	..	≠	..	..	≠	..	..	≠	..	..	E	II
H	3	(+)	(+)	..	+	+	..	..	..	..	+	(+)	..	..	E	II
„	5	(+)	+	+	≠	≠	(+)	+	+	..	≠	+	(+)	..	Da	II
„	28	+	..	..	≠	+	..	≠	..	..	..	..	..	..	Da	II
I	4	+	..	..	≠	≠	..	+	..	..	≠	+	..	..	Da	Tuberculous meningitis
J	4	≠	+	+	≠	≠	≠	≠	+	+	≠	≠	≠	..	Da	IV
K	?	..	≠	+	..	+	..	..	+	..	..	(+)	..	..	Da	I
L	6	≠	≠	≠	+	+	..	≠	≠	+	+	..	..	..	Da	I
M	7	≠	+	+	≠	(+)	..	≠	+	..	≠	(+)	..	..	Da	I
N	10	≠	≠	≠	+	(+)	..	≠	≠	≠	≠	+	..	..	Da	I
O	4	≠	(+)	..	≠	+	..	≠	(+)	..	≠	+	..	..	Da	II
P	5	≠	≠	≠	≠	+	..	≠	+	..	≠	≠	..	..	Da	I
Q	?	(+)	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	? C.S.F.
R	„	≠	≠	..	+	..	..	≠	≠	..	≠	+	..	..	Da	I
„	„	≠	≠	..	(+)	..	..	≠	(+)	..	+	..	..	..	E	I
S	36 hrs.	+	..	..	+	(+)	..	+	..	..	+	..	..	..	Da	I
T	?	+	..	..	(+)	(+)	..	(+)	..	..	..	..	..	..	Da	II
U	?	+	+	+	+	+	(+)	..	..	..	..	..	..	..	Da	III
V	?	+	(+)	(+)	+	+	+	..	..	..	..	..	..	..	Da	II
W	?	(+)	..	..	+	+	..	+	..	..	(+)	(+)	..	..	E	Meningococcus not isolated.
X		≠	+	(+)	(+)	(+)	(+)	..	..	..	..	..	..	..	D ι	Tuberculous meningitis.
Y	8	+	(+)	..	(+)	..	..	(+)	..	..	..	..	..	..	Da	Meningococcus not isolated.
Z		..	+	(+)	..	(+)	..	..	+	(+)	..	..	..	..	Da	I
A 1		..	..	..	+	(+)	(+)	..	..	..	..	..	..	..	E	I

≠ = complete absorption of complement. + = moderate absorption of complement. (+) = slight absorption of complement.
In the column referring to the form of antigen used, E = suspension of meningococcus, and Da = dissolved antigen as previously described.

Serum.	1 : 50	1 : 100	1 : 200	1 : 50	1 : 100	1 : 200	1 : 50	1 : 100	1 : 200	1 : 50	1 : 100	1 : 200	control. 1 : 50	Antigen used.	Disease.
1	..	..	..	(+)	..	..	..	..	..	..	..	..	..	E	Syphilis (II)
2	(+)	..	..	..	..	..	..	..	..	..	..	..	..	E	Syphilis (II)
3	..	..	..	..	..	..	..	..	..	..	..	..	..	E	Gonorrhoea
4	..	..	..	..	..	..	..	..	..	..	..	..	..	E	Gonorrhoea
5	..	..	..	..	..	..	..	..	..	..	..	..	..	E	Gonorrhoea
6	(+)	..	..	..	..	..	..	..	..	..	..	..	..	E	Gonorrhoea
7	..	..	..	..	..	..	..	..	..	..	..	..	..	E	Gonorrhoea
8	(+)	..	..	(+)	..	..	(+)	..	..	(+)	..	..	..	Da	Gonorrhoea
9	(+)	..	..	(+)	..	..	(+)	..	..	+)	..	..	..	Da	Gonorrhoea
10	(+)	..	..	(+)	..	..	(+)	..	..	(+)	..	..	..	Da	Syphilis
11	(+)	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	Syphilis
12	(+)	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	Syphilis
13	(+)	..	..	+	..	..	(+)	(+)	..	+	..	..	..	Da	Syphilis
14	(+)	..	..	(+)	+	..	(+)	(+)	..	(+)	..	..	..	Da	Syphilis
15	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Syphilis
16	(+)	..	..	(+)	(+)	..	(+)	..	..	(+)	..	..	..	Da	Syphilis
17	(+)	..	..	(+)	..	..	(+)	..	..	..	..	..	..	Da	Syphilis
18	..	..	..	+	..	..	(+)	..	..	..	..	..	..	Da	Syphilis
19	..	..	..	+	(+)	..	..	..	..	..	..	..	..	Da	Syphilis
20	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Syphilis
21	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
22	..	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
23	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
24	..	..	..	..	..	..	..	..	..	(+)	..	..	..	Da	Gonorrhoea
25	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
26	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
27	(+)	..	..	(+)	..	..	(+)	(+)	..	+	..	..	..	Da	Gonorrhoea
28	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
29	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
30	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
31	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Gonorrhoea
32	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Syphilis
33	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Syphilis
34	+	..	..	≠	+	..	(+)	..	..	≠	..	..	..	Da	Neg. Wassermann ; disease not determined
35	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Neg. Wassermann
36	..	..	..	≠	(+)	..	≠	..	..	≠	..	..	..	Da	Strongly pos. Wassermann
37	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	Neg. Wassermann
38	..	..	..	(+)	..	..	(+)	..	..	..	..	..	..	Da	Tubercle lung
39	..	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	Tubercle lung
40	..	..	..	(+)	..	..	(+)	..	..	..	..	..	..	Da	Strongly pos. Wassermann
41	(+)	..	..	(+)	..	..	(+)	..	..	..	..	..	..	E	Tubercular meningitis

≠ = complete absorption of complement. + = moderate absorption of complement. (+) = slight absorption of complement.

(iv) *Control Observations with the Serum of Patients suffering from Diseases other than Cerebrospinal Fever.*

Specimens of serum were collected from a series of control cases, and examined in the same way as the serum from cases of cerebrospinal fever. The results are recorded in the table on p. 73.

Of these 41 control cases, consisting of 18 cases of gonorrhoea, 17 of syphilis, 3 of tubercular infection, and 3 undetermined, complement was not fixed in any degree in 17 cases in the presence of the four type antigens, only very slightly in 22 cases, but in a marked degree in the two remaining, viz. 34 and 36. The tests for these two were done on the same day as 33–41 inclusive.

One, 34, was never diagnosed; 36 was a syphilitic serum. If these tests were accurate, other infections than that of the meningococcus may occasionally give positive results with the complement fixation test. See Cases I and X in the table dealing with cases of cerebrospinal fever.

(v) *Serum from Chronic Meningococcus Carriers.*

In order to complete the present observations, serum from a number of chronic meningococcus carriers was examined in the same way with results recorded in the following table:

It would seem that while antibodies capable of fixing complement in the presence of the meningococcus are rather more abundant in the serum of chronic carriers than in that of the control cases examined previously; and while occasionally the serum of a carrier reacts best in the presence of the type of meningococcus carried by him; nevertheless, on the whole, the complement-fixing capacity of the serum of chronic meningococcus carriers in presence of antigens of that micro-organism would appear to be somewhat lacking both in intensity and also in specificity.

It is of interest to note that Gates has recently observed that the serum of chronic carriers may show a higher agglutinin content for the meningococcus than that of normal persons.

Summary.

1. As was to be expected, complement-fixing antibodies were found to be far more abundant than agglutinins in the antimeningococcus sera examined.

2. In the monotypical therapeutic sera, complement-fixing antibodies were more abundant for the homologous type of meningococcus than for other types.

3. In case of the type agglutinating sera, this monotypical character of the chief complement-fixing antibodies present was still more marked.

4. After the fourth day of the disease, the serum of patients suffering from cerebrospinal fever contains complement-fixing antibodies for the meningococcus in fair abundance; and this complement-fixing reaction in some cases may serve as a guide to the type of meningococcus infecting the case.

SERUM OF CHRONIC CARRIERS

No. of case.	1			2			3			4			*Serum control.*	*Antigen used.*	*Type of meningococcus carried.*
	1 : 50	*1 : 100*	*1 : 200*	*1 : 50*	*1 : 100*	*1 : 200*	*1 : 50*	*1 : 100*	*1 : 200*	*1 : 50*	*1 : 100*	*1 : 200*			
A	≠	+	(+)	≠	≠	(+)	≠	(+)	..	..	..	..	..	Da	II
B	+	+	(+)	≠	+	+	+	+	..	..	..	..	..	Da	II
C	+	..	..	≠	+	+	+	(+)	..	(+)	..	..	..	Da	III
D	≠	+	..	..	..	..	(+)	..	..	..	..	..	..	Da	II
E	+	..	..	≠	(+)	..	+	..	..	..	..	..	..	Da	
F	+	+	..	+	(+)	..	+	(+)	..	+	..	..	..	Da	
G	..	+	..	..	≠	+	..	(+)	..	(+)	..	..	..	Da	
H	..	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	II
I	..	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	I
K	..	..	..	..	..	..	..	..	..	..	..	..	..	Da	III
L	..	..	..	(+)	(+)	..	(+)	..	..	(+)	..	..	..	Da	I
M	..	..	..	+	..	..	..	..	..	..	..	..	..	Da	III
N	(+)	..	..	(+)	..	..	..	..	..	..	..	..	..	Da	II

5. The serum of the majority of the control cases examined was relatively free of complement-fixing antibodies to the meningococcus of whatever type.

6. The serum of chronic carriers showed rather more of these antibodies than the control cases, but less than the serum of actual cases of cerebrospinal fever.

14. The Capacity of Antimeningococcic Serum for inducing Phagocytosis.

By W. J. Tulloch, O.B.E., M.D., Brevet-Major R.A.M.C.

A study of immunology in its relation to the meningococcus would be incomplete if the ' opsonic ' properties of sera prepared by inoculation of ' type ' strains of that micro-organism were not made the subject of inquiry.

It is essential that the relationship between the protective action, agglutinating properties, and capacity for inducing phagocytosis possessed by antimeningococcic sera, should if possible be determined. Such information is especially necessary in the case of sera prepared for therapeutic use.

Tests based on phagocytosis have been employed by Neufeld, and also by Flexner and Jobbling, to standardize antimeningococcus serum destined for the treatment of meningococcal infections; but these tests were performed with polyvalent sera for the most part, and no note was taken of the serological ' type ' of the coccus used in the tests.

An attempt was therefore made to determine the ' phagocyte-stimulating activity ' of sera specific to the types in respect of homologous and heterologous type strains of the meningococcus.

Investigations of other micro-organisms of known pathogenic significance has shown that several factors must be considered in the mechanism of infection, and therefore all these possible factors must be borne in mind in preparing and standardizing sera which are to be employed either for prophylaxis or therapeusis of the natural infection.

Thus an investigation of serological type strains of *B. tetani* has shown that :

1. The ' exotoxins ' of all the serological types of *B. tetani*, when inoculated into animals, call forth a group response : i. e. tetanus antitoxin prepared by inoculating the toxin (exotoxin) of one type strain of the bacillus, will neutralize with apparently equal potency the ' exotoxins ' of both serologically homologous and heterologous strains of the bacillus.

2. Inoculation of washed (broth) cultures of *B. tetani* results in the production of sera which possess high agglutinin titre specific for each type. Such sera are unable to induce phagocytosis of unwashed broth cultures, but do bring about marked phagocytosis of washed cultures of *B. tetani*. This phenomenon is definitely specific to the types : i. e. a serum prepared by inoculation of washed Type I *B. tetani* will bring about phagocytosis of washed Type I bacilli, but not of the other type bacilli, nor of unwashed Type I bacilli.

3. Inoculation of young unwashed broth cultures produces sera which have, in addition to high agglutinating value, a marked capacity for inducing phagocytosis of unwashed broth cultures. This activity is markedly specific, i. e. a serum prepared from a Type II culture will exhibit phagocytic properties markedly active in respect of only Type II cultures, as determined by agglutination.

From this we must conclude that cultures of *B. tetani* contain at least three antigens:

(*a*) An ' exotoxin ' which is not specific to the types.

(*b*) The bacilliary substance itself, which is specific to each type.

(*c*) An antiphagocytic substance present in young fluid cultures which is also specific in case of each type.

As similar factors might play a part in the pathogenesis of meningococcal infections, the following points deserve consideration:

I. Does the meningococcus, during its growth in artificial media, develop antiphagocytic properties ?

II. If so, are these specific for each serological type ?

III. Is the opsonic activity of a monotypical serum specific in relation to the type of meningococcus used for its preparation ? i. e. from the standpoint of production of ' opsonins ' are the type cocci, as distinct from their products, specific ?

IV. Is there any demonstrable relationship between the agglutinating activity and the ' opsonic ' activity of antimeningococcus serum ?

V. Can the results of phagocytic tests or agglutination tests be correlated with the antiendotoxin test on the one hand, or with the therapeutic efficacy of the sera on the other ?

At the outset I would call attention to the fact that these questions so far remain unanswered because unforeseen difficulties of technique were encountered, and up to the time of writing have not been overcome.

A. *Technique.*

Representative type strain cultures of the meningococcus obtained from the theca were grown on ' trypagar ' at 37° C. for 16 hours. They were then washed off with saline and standardized by the opacity method to contain approximately ten thousand million cocci per c.c. These suspensions were employed heated or unheated according to the nature of the particular experiment in which they were used.

Human white cells were prepared exactly according to the method advised by Wright, but no effort was made to work with ' leucocyte cream ' ; in place of so doing the product of the final centrifugalization was well mixed, and the whole used as a thick ' blood cream '.

The sera to be examined were heated to 60° C. for 30 minutes before being used in the tests, provided that the samples were not carbolized ; but if carbolized, the sera were kept till such time as the non-specific ' opsonizing ' properties of the serum had disappeared or become so markedly reduced that they did not interfere with the tests.

The sera were used in a range of dilutions of 1 : 1 to 1 : 100 and the test was carried out, using a modified Neufeld method as follows :

1/50 c.c. of serum (usually diluted).

1/50 c.c. culture suspension.

These were well mixed and incubated for 30 minutes at 37° C.

2/50 c.c. ' blood cream ' was then added, the whole well mixed, and incubated for a further period of 15 minutes.

Films were then made, dried in air, fixed with ' ether-alcohol-$HgCl_2$ mixture and stained with either ammonium carbonate methylene blue, or 1 : 10 carbol fuchsin.

It will be seen that the concentration of serum in each tube was 1 : 4 of that actually employed as a constituent of the mixtures.

Counts were then made of 200 leucocytes in each preparation using 1 : 12 objective, and a No. 4 eye-piece, while special care was taken critically to illuminate the slide.

B. *Estimation of the Activity of Serum.*

This estimation was made by noting in what dilution of anti-meningococcus serum the degree of phagocytosis was the same as that in presence of undiluted normal serum from the same species of animal as was used for the preparation of the immune serum.

No attempt was made to determine the number of micro-organisms each cell took up, but in order to have some comparison between one serum and another, the percentage of actively phagocytic cells in each preparation was determined ; cells containing a large number of cocci were designated ' positive ' while those containing none or few—2 to 6—were designated ' negative '. In this way a percentage value of positive cells was obtained for each preparation and so the dilution of specific serum which gave the same degree of phagocytosis as did undiluted normal serum was ascertained.

I. Does the meningococcus develop antiphagocytic qualities during its growth ?

II. If so, are these specific in case of each serological ' type ' ?

Two series of experiments were conducted with a view to investigating these points.

First series of Experiments. In the first series the following procedure was adopted : Cultures of a Type I strain and of a Type II strain of meningococcus were grown for periods of 8, 10, and 16 hours and complete crossed experiments were carried out, using suspensions of these cocci in presence of a number of dilutions of Type I and Type II antimeningococcus (horse) serum.

These sera were used in the experiments in dilutions of 1 : 10, 1 : 20, and 1 : 40, giving final dilutions of 1 : 40, 1 : 80, and 1 : 160.

Negative results only were obtained in this series of experiments, but a point of some importance was observed. In these tests it was found that the Type I strain that was used was not phagocytosed in presence of any serum while the Type II strain was phagocytosed in presence of all the dilutions of both antimeningococcus sera of both ' types ' and in presence of 1 : 10 normal serum.

Second series of experiments. In series 1 it was seen that cocci appeared to vary greatly in their susceptibility to phagocytosis.

It was therefore decided for the time being to limit attention to Type I cocci, as it was possible that the complete absence of phagocytosis in the case of Type I in the first series might have been due to an antiphagocytic property possessed by certain strains of cocci in the fresh state.

In the first place a number of Type I antimeningococcus sera were gone over until one was obtained which gave a slight degree of phagocytosis in presence of the same strain of Type I that was used in the previous series of experiments.

A suspension obtained by washing off a 16-hour culture of this coccus in saline was standardized to contain ten thousand million cocci per c.c. This suspension was then divided into two portions—'X' and 'Y'. 'X' was used in the test without further preparation, while 'Y' was heated to 60° C. for 30 minutes before it was employed.

Each of these ('X' and 'Y') was then tested in presence of the Type I serum which had been found to exhibit a slight phagocytic activity. Type II serum was also used in the test to serve as a control for the observations made with the Type I serum.

The results were encouraging in that the Type I serum produced a certain amount of phagocytosis, while the controls both with normal serum and Type II serum did not.

Both emulsion 'X' (unheated) and 'Y' (heated) were however taken up with equal avidity by the leucocytes in presence of the Type I serum.

Owing to the hypersusceptibility of most Type II cocci to phagocytosis, the converse experiment could not be carried out.

The experiment was repeated several times with various Type I and II cocci after culture on both solid and fluid media, but in no instance were the results obtained of such a nature that definite evidence was forthcoming that the meningococcus developed antiphagocytic properties in its growth—at least thermolabile antiphagocytic properties.

These experiments, although they failed to solve the problem under consideration, were valuable, in that they called attention to certain points of importance in the further conduct of the research.

Thus they showed that the meningococcus is extremely variable in its susceptibility to phagocytosis. One strain may prove refractory on one occasion, and a few days later it may be readily phagocytosed in presence of one and the same serum.

For this reason it was obvious that the subjects involved in questions 3, 4, and 5 could not be satisfactorily studied by the 'opsonic' method and any results obtained could only be of doubtful value.

The only conclusion that could be drawn from the experiments of the two series was that no evidence was obtained that the meningococcus developed antiphagocytic substances in its growth.

Question II therefore remains uninvestigated.

III. Is the 'opsonin' activity of antimeningococcus serum specific in relation to the type of coccus used for its preparation?

A series of experiments was set up with a view to the investigation of this problem.

It is to be noted that in this series and in all subsequent phases of the present investigation 16-hour cultures were used, and all were heated to 60° C. for 30 minutes before carrying out the experiments. The reason for adopting this slight modification of technique was that the tests, so far made, indicated that cocci heated to 60° C. for 30 minutes reacted in the tests as did fresh cocci, and appeared

to be slightly less liable to undergo intracellular digestion, while autolysis of the cocci—another factor that made microscopical observation difficult and unsatisfactory—was somewhat reduced.

In this, as in the previous series of tests, the variability of the cocci in their susceptibility to phagocytosis rendered the work extremely laborious, and always left in doubt what interpretation should be made of the results obtained.

In a series of over 20 crossed experiments with a number of dilutions of serum in each test and using Type I and Type II cocci in presence of monotypical Type I and Type II sera, specific results in relation to type were obtained on two occasions only. With the four dilutions of serum employed this involved 32 counts—16 on each occasion—which were perfectly clear cut.

Notwithstanding this definite evidence of specific 'opsonic' property possessed by the sera, the findings are of really no practical value, for the method is too uncertain to permit of its employment for the standardization of serum, until at least we know what are the conditions which alter so markedly the susceptibility of the cocci to phagocytosis.

From this series of experiments it was concluded that there was some slight evidence of specific relationship between the type coccus used for immunization and the 'opsonic' activity of the serum obtained. The evidence was, however, insufficient to constitute a definite answer to the question at issue.

IV. Is there any relationship between the agglutinating activity and the 'opsonizing' activity of antimeningococcus serum?

In this series of tests it was shown that a serum of but low agglutinating titre might yet be fairly active in inducing phagocytosis. Here again a fallacy is introduced by the extreme variability of the cocci in their susceptibility to phagocytosis.

When the converse of this finding was examined one failed to obtain evidence that sera of high agglutinating titre might not induce phagocytosis.

The difficulty met with here was that all sera exhibiting a high agglutinating titre produced, to a greater or less extent, flocculation in the tubes used for the phagocytic tests. The white cells in the preparation were found mainly in the clumps of cocci, and in such circumstances it was found to be really impossible to determine whether phagocytosis had or had not occurred.

I therefore failed satisfactorily to demonstrate any relationship between agglutinating titre and 'opsonic' titre in the case of antimenigococcus serum.

V. Can the results of phagocytic or agglutination tests be correlated either with the 'mouse test' or with the therapeutic efficacy of antimeningococcus serum?

A large number of experiments were performed in the investigation of this problem and the following is a synopsis of the results obtained:

(*a*) In making this inquiry I was fortunate enough to obtain a sample of serum which had given consistently good results in the treatment of cerebrospinal fever. This serum had been examined by Lt.-Col. Gordon by the 'antiendotoxin' test, and was found to give definite protection to mice.

Its ' opsonic ' properties were examined several times and were found to be by no means marked, while its agglutinin titre was low.

This finding suggests that a serum may possess protective properties, and may prove valuable in the treatment of the natural disease even although it is deficient in agglutinating or ' opsonizing ' qualities.

(*b*) On the other hand, sera which had active ' opsonic ' properties not infrequently failed to pass the ' antiendotoxin ' test.

[It is to be noted that those specimens of serum which did pass the ' antiendotoxin ' test have on the whole proved satisfactory in the treatment of the natural disease.]

(*c*) Certain sera were shown to possess—so far at least as one could investigate them—antiendotoxic, phagocytic, and agglutinating properties. There is, however, so far no evidence that these proved of special value in the treatment of cerebrospinal fever. As so few samples of serum possess at the same time antiendotoxic, phagocytic, and agglutinating properties, it is doubtful whether the waste could be borne were we to accept for therapeutic use only those sera which did exhibit all three properties.

The following general conclusions seem permissible from this section of the work :

1. The results fail to show the relationship which the ' mouse ' test bears to the agglutinating and ' phagocytosing ' properties of the serum.

2. In the present state of our knowledge the ' mouse test ' appears to be the most satisfactory method which we have for standardizing antimeningococcus serum. Agglutination and phagocytosis tests cannot be regarded as in any way satisfactory for this purpose.

C. *Discussion.*

The findings of these investigations are, in the main, of negative value only, and in view of the almost insurmountable difficulties of technique which were encountered, the above statements are made with reserve.

It could, of course, be argued that by the choice of suitable type strains, the ' opsonic ' test might be made sufficiently accurate to give some kind of an indication of the probable therapeutic value of a serum. I failed, however, to obtain cocci which over periods longer than three to four days gave consistent results ; if then a phagocytosis test is to be used for the standardization of antimeningococcus serum, the basis for comparison of one serum with another must be a serum of already known therapeutic efficacy.

15. Lysin.

A. *The Literature concerning Lysin to the Meningococcus.*

The first to draw attention to the fact that fresh normal human serum is bactericidal to the meningococcus was David Davies (1905), who observed a difference between the lytic power of the serum of various normal individuals, and found that this property was definitely increased in the case of serum from patients undergoing

an attack of cerebrospinal fever. He observed also that the meningococcus was destroyed by the fresh serum in 1–2 hours at 37° C., and that the bactericidal power of the serum was diminished, but not entirely destroyed, by heating it to 60° C. for 30 minutes. The medium used by Davies when carrying out these observations was ascitic broth.

In the following year Jochmann (1906), during an investigation of the mode of action of antimeningococcus serum, observed that whereas the serum by itself has no destructive action on the meningococcus, it becomes actively lytic when mixed with complement derived either from the rabbit or guinea-pig. The medium used by Jochmann was ascites fluid.

In course of his studies of the meningococcus, Flexner (1907) drew attention to the rapid disappearance of meningococci when introduced into the peritoneal cavity of the guinea-pig, and concluded that their rapid destruction could be effected without the direct intervention of phagocytes. He found that both the serum and peritoneal exudate of the guinea-pig had a lytic action on the meningococcus *in vitro*, and in case of the peritoneal fluid this lytic power was still present little, if at all, impaired after the fluid had been heated to 60° C., or had stood for two weeks at 0° C. He attributed this action of the fluid to the presence of an injurious substance liberated from the destroyed leucocytes and acting on the cocci in such a manner as to bring them under the solvent influence of their own autolytic enzyme. Flexner noticed also that the more virulent meningococci resisted disintegration in the peritoneal exudate more than the less virulent ones.

The next observer to study the lytic effect of antimeningococcus serum on the meningococcus was Dopter (1910), who approached the subject from a new direction. Having found in conjunction with Biot that horses in process of immunization against the meningococcus are liable shortly after receiving an intravenous injection of meningococci to develop symptoms resembling those of anaphylactic shock, he proceeded to investigate the phenomenon *in vitro*. The growth of a 24-hours' culture of the meningococcus in a Roux flask was suspended in 20 c.c. of saline and 1·5 c.c. of this suspension was mixed with 1 c.c. of fresh antimeningococcus serum from a susceptible horse and injected intravenously into a guinea-pig. The guinea-pig at once developed symptoms of anaphylactic shock and died in a few minutes. Only fresh antimeningococcus serum produced this effect when mixed with the cocci; the same serum after being heated to 55° C., or normal serum, or antiplague serum, all proving inert. Dopter attributed the phenomenon to the liberation of toxin from the cocci by the action of lysin present in the fresh antimeningococcus serum. Comparative observations in the same way with *M. catarrhalis*, *M. cinereus*, *M. siccus*, parameningococci, and gonococci taught him that this action of fresh antimeningococcus serum was more specific and complete for the meningococcus than for the other cocci. There was, however, a minor degree of toxicity when the antimeningococcus serum was mixed with some of these other cocci, which effect he proved, on applying the saturation test, to be due to the presence of co-lysins in the serum comparable to the co-agglutinins and co-

precipitins which he had previously demonstrated in antimeningococcus serum.

Armed with this new knowledge, Dopter now proceeded to inquire whether the Pfeiffer Reaction, so well known in relation to the identification of the cholera vibrio and the typhoid bacillus, does not also serve to distinguish the meningococcus from parameningococci. On the day preceding the test 1 cc. of fresh antimeningococcus serum was injected into the peritoneal cavity of a guinea-pig of 250 grammes weight. Twenty-four hours later a slope culture of the micro-organism to be tested was suspended in 6 c.c. of saline, one c.c. of this injected into the peritoneal cavity of the guinea-pig, and 20 and 30 minutes later films made of the peritoneal fluid, stained, and examined microscopically. A control experiment showed that the meningococcus homologous to the serum disappeared in this time, whereas the parameningococci and other cocci simulating the meningococcus did not do so to anything like the same extent. By applying once again the absorption method, and saturating the serum with the various cocci before injecting it, Dopter succeeded in proving that the diminution which some of these cocci other than the meningococcus are apt to undergo in the presence of antimeningococcus serum in the peritoneal cavity of the guinea-pig is due to the action of co-lysins, since the cocci in question were found to absorb the lysins for themselves from the sera, but to leave the specific lysin for the meningococcus unaffected. As a further result of his study of the lysin present in antimeningococcus serum with the absorption test, Dopter confirmed the plurality of parameningococci—a fact previously demonstrated by him when working on similar lines with agglutinins.

B. *Object of the Present Investigation.*

It would appear from the literature, therefore, that lysin for the meningococcus occurs in normal human serum; and from the illuminating work of Dopter it would seem that the lysin present in antimeningococcus serum is differentiated into specific lysin and co-lysins in similar manner to the agglutinin. While from the observations of Flexner of the fate of the meningococcus when introduced into the peritoneal cavity of the guinea-pig, the activity of lysin would appear to form a prominent feature in the defence of the normal animal, it would seem that the precise part played by this antibody in immunity to the meningococcus, and in particular its relative importance in relation to the therapeutic potency of anti-meningococcus serum are matters still unknown. As these are the very points upon which information is most required at the present time, and they cannot be satisfactorily settled until a suitable method has been obtained for measuring the amount of lysin to meningococci of one or another type in a sample of serum or exudate, the first object of the investigation now to be described was to work out, if possible, a method of determining *in vitro* the quantity and quality of lysin in antimeningococcus serum. The steps by which such a method has been sought are described in the following section, and also the result of an investigation of the lysin content of three

samples of antimeningococcus serum, one of which had been found clinically to possess an exceptionally high degree of potency, while the others had but small therapeutic value.

C. *Studies with a View to obtaining a Method of estimating Lysin* in vitro.

(i) *Apparatus, &c.* The same small tubes plugged with wool and the same water bath at 37° C. were used as when investigating the haemolytic substance and the reductase of the meningococcus. Falling amounts of the serum of test in the diluent to be described later were put up in these tubes in 0·5 c.c. amounts. Complement was either diluted directly in the serum of test, or was diluted and added separately ; in which case 0·1 c.c. of the requisite dilution of complement was added to each tube. Finally, the living coccus was also added to each tube ; it was obtained in the following way. A slope culture of the meningococcus was made and incubated for 18 hours at 37° C. The condensation fluid was then removed, replaced by 1 c.c. of the diluent, and the growth suspended in the latter by raking it off with a wire. Next 0·9 c.c. of diluent was placed in each of four small tubes, 0·1 c.c. of the suspension of the slope culture was added to the first of these and, after shaking, 0·1 c.c. transferred to the next, and the dilution continued in the same way through the other two tubes. One-tenth of a c.c. of the fourth tube thus contains 0·00001 of the slope culture, which is a suitable amount of the coccus to add to each of the tubes holding falling dilutions of the serum and the requisite amount of complement. After adding the coccus, the tubes were well shaken and the racks holding them were then placed in the water bath at 37° C., where they remained for two hours, being occasionally shaken. At the end of this time the whole of the contents of each tube were transferred to tubes containing 15 c.c. of trypagar already melted and cooled to 45° C., in a water bath, and plates poured. The plates were then incubated at 37° C. and examined after 24 and 48 hours. The pipettes used to transfer the contents of the tubes to the melted trypagar were operated by a rubber teat and were sterilized before use by wrapping them in a clean towel and steaming them for 30 minutes in a small steamer. This was found to be a far more efficient method of sterilizing pipettes than heating them in a copper box.

(ii) *Diluent.* A great deal of difficulty was met with before a satisfactory diluent could be found. At first ascites broth was used, but the results were irregular owing to the tendency of the meningococcus to decrease during its exposure in this medium for two hours at 37° C. Samples of ascites fluid that had been repeatedly heated to 55° C. were next tried ; but they also proved unsatisfactory for a similar reason. These specimens of ascites fluid had been preserved by adding chloroform to them in the first place, and it is conceivable that this had rendered them unsuitable for the present purpose. So serious was this difficulty of a suitable diluent that the research was on the point of being abandoned when it was decided to try inactivated normal rabbit's serum. Details of this experiment are shown below. Two samples of ascitic fluid were tried at the same time for purposes

of comparison. As the diluent sought for must not only preserve the life of the meningococcus without any loss during two hours at 37° C., but must also be suitable for demonstrating the action of lysin, the effect was tried at the same time of adding falling amounts of fresh guinea-pig complement.

Particulars of this experiment are as follows :

No.	*Diluent.*	*Complement.*	*Growth.* 1 *Day.*	2 *Days.*
1	Ascites No. 1	1 : 5	2 colonies	2
2	,, ,,	1 : 10	0	0
3	,, ,,	1 : 20	0	1
4	,, ,,	..	8 colonies	312
5	Ascites No. 2	1 : 5	12 colonies	30
6	,, ,,	1 : 10	0	0
7	,, ,,	1 : 20	0	1
8	,, ,,	..	5 colonies	17
9	Normal rabbit serum	1 : 5	0	2
10	,, ,, ,,	1 : 10	0	0
11	,, ,, ,,	1 : 20	0	0
12	,, ,, ,,	..	Uncountable	Uncountable
13		Undiluted	0	,,
14		1 : 10	2 colonies	5

The result of the controls with the three media alone, namely Nos. 4, 8, and 12, showed that the normal rabbit serum was a far more suitable medium for the meningococcus for the present purpose than the specimens of ascitic fluid. From the bactericidal action evident in Nos. 9, 10, and 11, it was also clear that the normal rabbit serum was suitable for demonstrating the action of lysin. Inactivated normal rabbits' serum, therefore, was adopted for routine use as a diluent.

(iii) *The Degree to which the Meningococcus is lysed in Vitro by normal Rabbit Serum when activated by fresh Guinea-pig Complement.* Having obtained a promising diluent, namely normal rabbit serum inactivated and sterilized by repeatedly heating it to 55° C., the following experiment was made in order to determine the degree of lytic action of 1 part of fresh guinea-pig complement in 20 parts of this diluent. The test was made at the same time on two different meningococci, namely, Type I, Smith, and Type II, Lindenbaum. A slope culture of each coccus was suspended in 1 c.c. of normal rabbit serum and a series of decimal dilutions made from each in 0·9 c.c. amounts of the serum ; 0·1 c.c. of each of these falling dilutions of the culture was then added to tubes holding 0·5 c.c. amounts of the 1 : 20 dilution of complement in normal rabbit serum, and placed for two hours at 37° C. The result was as follows :

No.	*Meningococcus.*	*Amount of Slope.*	*Result.*
1	T. I, Smith	0·1	Good growth
2	,,	0·01	No growth
3	,,	0·001	,,
4	,,	0·0001	,,
5	,,	0·00001	,,
6	T. II, Lindenbaum	0·1	Good growth
7	,,	0·01	12 colonies
8	,,	0·001	No growth
9	,,	0·0001	,,
10	,,	0·00001	,,

Controls of Nos. 5 and 10 in the normal rabbit serum without complement gave in both cases good growth. It will be observed that when complement was present, 0·01 of a slope and lesser amounts were practically destroyed. If the average total growth on a one-day trypagar slope be taken as 20,000 million cocci, it follows that approximately 200 million meningococci were destroyed in two hours at 37° C. by half a cubic centimetre of normal rabbit serum containing one-twentieth part of normal guinea-pig complement.

(iv) *The Rate at which the Meningococcus is lysed in Vitro by normal Rabbit Serum when activated by fresh Guinea-pig Complement.* Two separate concentrations of complement were used, namely 1 : 20 and 1 : 50. The meningococcus employed was Type II, Lindenbaum, and the dose 0·00001 of a slope. The experiment was carried out in triplicate, one set of tubes being cultivated after half an hour at 37° C., the next after one hour, and the third after two hours at this temperature. Both concentrations of complement gave the same result. There was but little diminution of the cocci in 30 minutes ; in 60 minutes about half of them had been destroyed ; and in two hours practically all of the cocci were dead. It would seem, therefore, that two hours at 37° C. is a suitable exposure to give when carrying out lytic tests on the meningococcus *in vitro.*

(v) *Effect of Age on the Lytic Power of Complement.* Complement from the guinea-pig was now tested after standing at laboratory temperature for one day, two days, and three days respectively. As before, the diluent used was inactivated normal rabbit serum. The coccus added was 0·00001 of a slope of Type II, Lindenbaum. The result was as follows.

[In this and the following experiments the sign O = no growth ; (+) = a few colonies only ; + = a fair number ; ≠ = colonies more numerous still ; and +++ = full growth.]

No.	*Age of Complement.*	*Concentration.*	*Growth.*
1	1 day	1 : 10	O
2	,,	1 : 50	O
3	,,	1 : 100	(+)
4	,,	1 : 200	≠
5	2 days	1 : 10	O
6	,,	1 : 50	O
7	,,	1 : 100	≠
8	,,	1 : 200	+++
9	3 days	1 : 10	+
10	,,	1 : 50	+
11	,,	1 : 100	≠
12	,,	1 : 200	+++

The complement, therefore, remained active for two days, but after that underwent a considerable diminution in lytic power.

(vi) *The Effect of Heat on the Lytic Power of Complement.* It was found that when a mixture of normal rabbits' serum and guinea-pig complement was heated to 55° C. for 30 minutes the lytic action on the meningococcus was abolished.

(vii) *Comparison of the Lytic Power for Type* II *Meningococcus of fresh Serum from a Normal and Prepared Guinea-pig respectively.* A guinea-pig weighing 300 grammes having received two months

previously an intraperitoneal dose of 40,000 million living meningococci (Type II, Lindenbaum) and recovered after several days' illness, this animal was given a dose of 10,000 million of this same coccus, and two days later the lytic power of its serum was compared with that of a normal guinea-pig. As before, inactivated normal rabbits' serum was used as diluent. The dose of coccus added was 0·00001 of Type II, Lindenbaum. The result was as follows :

No.	*Material.*	*Concentration.*	*Growth.*
1	Normal G.P. serum	1 : 50	0
2	,, ,,	1 : 100	0
3	,, ,,	1 : 200	0
4	,, ,,	1 : 500	≠
5	,, heated to 55° C.	1 : 10	≠
6	Prepared G.P. serum	1 : 50	0
7	,, ,,	1 : 100	0
8	,, ,,	1 : 200	+
9	,, ,,	1 : 500	≠
10	,, heated to 55° C.	1 : 10	≠

The serum of the normal guinea-pig, therefore, was actually more lytic than that of the prepared animal in this experiment.

(viii) *Lytic Effect of Normal Rabbit Serum activated by Guinea-pig Complement upon the four Types of Meningococcus respectively.* This test was carried out simultaneously with four meningococci—one of each type. In all four instances the dose of living coccus used was 0·00001 of a slope. The results were as follows :

	I	II	III	IV
Complement.	*Smith.*	*Lindenbaum.*	*Chase.*	*Taplow.*
1 : 50	≠	0	≠	(+)
1 : 100	≠	(+)	≠	(+)
1 : 200	≠	≠	≠	≠
..	≠	≠	≠	≠

≠ = good growth of meningococcus.

The lytic action of this specimen of normal rabbit serum, therefore, when activated by guinea-pig complement, was chiefly on Type II and to a less extent on Type IV. The action on Types I and III was apparently nil. In experiment (iii), however, the specimen of normal rabbit serum employed, when activated by guinea-pig complement 1 : 20, was definitely lytic for Type I as well as for Type II ; and in some further experiments with complement 1 : 10 and 1 : 20 a lytic effect was obtained with normal rabbit serum upon Types III and IV. It would seem, therefore, that while the lytic action is generally more marked on Type II meningococci than on the others, normal rabbit serum, when activated by strong guinea-pig complement, may be lytic for all four types of the meningococcus.

(ix) *Absorption Tests.* Since normal rabbit serum when activated by complement from the guinea-pig was found to be lytic for the meningococcus—especially for Type II—this action could only be due to the presence of lysin. As the chief object of this investigation was to obtain a method of determining the quality and quantity of lysin in antimeningococcus serum, and normal rabbit serum was the best medium for such tests so far obtained, it was plain that this lysin must be removed from the normal rabbit serum by saturation with the coccus.

A preliminary experiment was made, therefore, to determine what dose of the coccus—killed by heating it to 55° C.—was needed in order to remove this lysin.

Experiment to determine the Dose of Type II Meningococcus necessary in order to remove the Type II Lysin from normal Rabbit Serum mixed with Guinea-pig Complement. Procedure was as follows : A dilution was made of 1 part guinea-pig complement in 25 parts of inactivated normal rabbit serum. This was now divided into six portions each of 2·5 c.c. which were transferred to centrifuge tubes. To the first of these tubes an equal quantity of normal rabbit serum was added, and to the others the same diluent containing in turn $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, and $\frac{1}{32}$ of the growth on a slope of Type II Lindenbaum, previously killed by heating to 55° C., for 40 minutes. These six centrifuge tubes were well shaken, placed for an hour and a half at 37° C., and after being centrifuged, the supernatant fluid was removed and tested for its lytic power on Type II Lindenbaum in the usual way. As a result it was found that whereas the control showed lysis up to a dilution of the complement of 1 : 200, the same fluid, after being saturated with $\frac{1}{4}$, $\frac{1}{8}$th, and $\frac{1}{16}$ of the one-day slope culture, had lost all lytic power for the coccus at a dilution of the complement of 1 : 50, though this lytic power was still present at this dilution in case of the fluid which had been saturated with $\frac{1}{32}$ of the slope. The conclusion drawn from these observations was that $\frac{1}{8}$ of the total growth on a one-day slope, killed by heating it to 55° C., was a suitable dose to use in order to absorb the lysin from 2·5 c.c. of a 1 : 25 dilution of complement in normal rabbit serum.

(x) *The Effect of Saturating Normal Rabbit Serum with Type I and Type II Meningococcus respectively.* A specimen of serum from the normal rabbit was inactivated by heat and divided into three portions, two of which were then saturated with Type I and Type II meningococci respectively, and the cocci removed by centrifuge. The lytic power of the serum before and after saturation was then determined on both cocci in the presence of complement. Only saturated serum, of course, was used to dilute the saturated serum after addition of complement. The results were as follows :

No.	*Diluent.*	*Complement.*	*Meningococcus.*	*Growth.*
1	Unsaturated	1 : 100	Type I	≠
2	Normal rabbit serum	1 : 200	Smith	≠
3	,, ,,	1 : 400	,,	≠
4	Do. after saturation with Type I	1 : 100	,,	≠
5	,, ,,	1 : 200	,,	≠
6	,, ,,	1 : 400	,,	≠
7	Serum unsaturated	0	,,	≠
8	Do. saturated	0	,,	≠
9	Unsaturated	1 : 100	Type II	0
10	Normal rabbit serum	1 : 200	Lindenbaum	0
11	,, ,,	1 : 400	,,	≠
12	Do. after saturation with Type II	1 : 50	,,	≠
13	,, ,,	1 : 100	,,	≠
14	,, ,,	1 : 200	,,	≠
15	Serum unsaturated	0	,,	≠
16	Do. saturated	0	,,	≠

It is seen that the unsaturated normal rabbit serum contained no lysin for Type I, but was lytic for Type II up to a dilution of 1 : 200. The Type II coccus removed this lysin very completely.

This experiment confirmed previous observations as to the tendency of normal rabbit serum to contain lysin for Type II meningococcus, and emphasized the necessity of saturating this serum with Type II meningococcus before using it as a diluent when determining the amount of Type II lysin in a sample of antimeningococcus serum.

(xi) *The Effect of Saturation with Types I* and *II respectively on the Type II Lysin present in Normal Rabbits' Serum.* The result of this experiment was as follows :

No.	*Material.*	*Complement.*	*Meningococcus.*	*Growth.* 1 *Day.*	2 *Days*
1	Unsaturated serum	1 : 50	Type II	0	0
2	,, ,,	1 : 100	Lindenbaum	0	1 colony
3	,, ,,	1 : 200	,,	0	5
4	Serum after saturation with Type I	1 : 50	,,	0	1
5	,, ,,	1 : 100	,,	0	3
6	,, ,,	1 : 200	,,	≠	≠
7	Serum after saturation with Type II	1 : 50	,,	≠	≠
8	,, ,,	1 : 100	,,	≠	≠
9	,, ,,	1 : 200	,,	≠	≠
10	Diluent	0	,,	≠	≠

≠ = Good growth of meningococcus.

It is seen that while the Type II coccus removed the Type II lysin from the serum completely, the Type I coccus only reduced it slightly. The lysin, therefore, would appear to be specific.

(xii) *Does fresh Guinea-pig Complement contain Lysin for the Meningococcus ?* In course of some experiments in which the inactivated normal rabbits' serum used as diluent had been saturated previously with the meningococcus of test, evidence was found that fresh guinea-pig complement is distinctly lytic for all the types of meningococcus when it is present in concentrations as high as 1 : 5 or 1 : 10, and even in some cases when more diluted.

Before examining the lytic power of a sample of antimeningococcus serum, therefore, it is necessary first of all to remove any lysin that may be present in the inactivated normal rabbits' serum to be used in the test as diluent. For this purpose it has been found advisable, especially in the case of Type II, to saturate such diluting serum twice over with the meningococcus which it is proposed to use. A rough guide to the amount of lysin present is afforded by the agglutinin index—when the added cocci are no more agglutinated it may be taken that sufficient of the lysin has been removed. Another precaution which is necessary is to avoid using too strong a dose of complement. Careful controls, with all the ingredients used, should, of course, be put up in each test.

(xiii) *Conclusions.* It would appear from these observations :

(1) That the presence in serum of lysin for the meningococcus can be demonstrated *in vitro.*

(2) That by using falling amounts of the inactivated serum of test,

and supplying a suitable dose of complement, it should be quite possible to make the test a quantitative one. So far as these observations go, normal rabbits' serum previously inactivated by heat, and freed by saturation of any lysin normally contained by it, would appear to be the best diluent to use.

(3) That as the complement of the normal guinea-pig has a lytic effect by itself upon the meningococcus when insufficiently diluted, it is necessary to avoid using too strong a dose of complement when determining the lytic activity of a sample of serum for the Meningococcus.

D. *Investigation of the Lysin Content of Samples of Antimeningococcus Serum.*

The three samples of antimeningococcus serum tested were specimens of monotypical serum prepared by Dr. Stanley Griffith, and the horses providing them had been immunized by intravenous injection of the raw coccus. These sera were in actual use therapeutically at the time when they were examined. The agglutinin content of these sera for both of two meningococci of the homologous type was over 1 : 4,000.

Experiment 1. Comparison of the Lytic Activity of two specimens of Type II Antimeningococcus Serum with that of Normal Horse Serum for Type II Meningococcus. (*a*) Preliminary saturation of the diluting serum. Twenty-five cubic centimetres of normal rabbits' serum inactivated by heating it to 55° C. was saturated on each of two occasions with the whole of the growth from a plate culture of Type II Lindenbaum, removed by suspending the growth in a few c.c. of the serum, and then killing the cocci by exposing the suspension for 30 minutes to 55° C. After contact on each occasion for 24 hours at room temperature, the fluid was centrifuged, the serum pipetted off from the deposited cocci, and heated for half an hour to 55° C.

(*b*) Dilution of the sera of test. A 1 : 50 dilution of fresh guinea-pig complement was now made in this saturated serum, and with this a series of falling dilutions prepared for each of the three sera of test. These dilutions represented 1 : 100, 1 : 500, 1 : 1000, and 1 : 2000 of each of these sera respectively, and bulked in all cases to 0·5 c.c.

(*c*) Controls. These consisted of (1) 0·5 c.c. of the saturated serum used as diluent ; (2) a dilution of 1 part of each serum of test in 50 of this diluent (in order to exclude any bactericidal action of the preservative in them) ; and (3) complement diluted 1 : 10, 1 : 50, 1 : 100, and 1 : 200 of the diluent.

(*d*) Dose of living coccus. The amount added to each tube was 0·00001 of a one-day slope culture of Type II Lindenbaum.

The results were as shown on p. 91.

It is seen that the complement by itself was bactericidal to the Lindenbaum Coccus at a concentration of 1 : 10 ; but this lytic power was not evident at 1 : 50, the concentration used in the test. Both samples of Type II serum showed some evidence of lysin for the coccus ; 33 being superior in this respect to 55. The Controls were all satisfactory.

No.	*Serum.*	*Conc.*	*Complement.*	*Growth.*
1	Normal horse	1 : 100	1 : 50	≠
2	,,	1 : 500	,,	≠
3	,,	1 : 1,000	,,	≠
4	,,	1 : 2,000	,,	≠
5	Type II Serum	1 : 100	,,	(+)
6	M.R.C. 33	1 : 500	,,	(+)
7	,,	1 : 1,000	,,	+
8	,,	1 : 2,000	,,	≠
9	Do.	1 : 100	,,	+
10	M.R.C. 55	1 : 500	,,	+
11	,,	1 : 1,000	,,	≠
12	,,	1 : 2,000	,,	≠
13	Diluent	..	..	≠
14	Normal horse	1 : 50	..	≠
15	Type II serum 33	1 : 50	..	≠
16	Type II serum 55	1 : 50	..	≠
17	Complement	..	1 : 10	3 colonies
18	,,	..	1 : 50	≠
19	,,	..	1 : 100	≠
20	,,	..	1 : 200	≠

This test was repeated on the following day with a similar result. The activity of the complement on this second occasion was as follows : 1 : 10 produced complete destruction of the coccus, but 1 : 20 was without obvious lytic effect. The dose used in the test was as before 1 : 50.

Experiment 2. Comparison of the Lytic Power of Type I, Type II, and Normal Serum respectively for Type I Meningococcus. In the present experiment the sample of Type I serum tested was No. 49a, and it was compared with the Type II serum No. 33, and also with the normal horse serum tested in the preceding experiment against Type II meningococcus. The normal rabbit serum used as diluent was saturated before use with the Type I strain—Cooper—here employed for the purpose of testing the lytic power of the sera.

The present test was put up in duplicate, using complement in the first series at a concentration of 1 : 25, and in the second at 1 : 50. As a Control of the complement alone, however, at 1 : 25 was found to produce destruction of the coccus, the first series was discarded.

No.	*Serum.*	*Conc.*	*Complement.*	*Growth.*
1	Normal horse	1 : 50	1 : 50	≠
2	,	1 : 200	,,	≠
3	Type I, No. 49a	1 : 50	,,	≠
4	,,	1 : 200	,,	≠
5	Type II, No. 33	1 : 50	,,	≠
6	,,	1 : 200	,,	≠
7	Diluent	..	..	≠
8	Normal horse	1 : 50	..	≠
9	Serum 49	1 : 50	..	≠
10	Serum 33	1 : 50	..	≠
11	Complement	..	1 : 10	0
12	,,	..	1 ; 25	0
13	,,	..	1 : 40	≠
14	,,	..	1 : 50	≠

The sera examined for lytic power in the present experiment were

tested in a dilution of 1 : 50 and 1 : 200 only. Controls were put up as before, and the dose of living coccus added to each tube was 0·00001 of a slope of Type I, strain Cooper. The results were as shown above.

Here the lytic power of the complement was well marked at 1 : 25, but had gone at 1 : 40. None of the sera tested with the addition of complement 1 : 50 showed any lytic effect at all.

Experiment 3. Comparison of the Effect of the Lytic Power of Guinea-pig Complement on Type I Meningococcus when diluted in Saturated Rabbit Serum, Normal Horse Serum, and Type I and Type II Anti-meningococcus Sera respectively. As it seemed possible that the failure to obtain evidence of lysin in the Type I serum in the last experiment might be due to the dose of complement there used having been too small, the present experiment was made in order to compare the lytic activity of complement when diluted in the sera mentioned above. The sera in question were all diluted 1 : 50 with normal rabbit serum so as to get beyond the zone of activity of any preservative contained by them, and the complement was then diluted in this solution.

Another strain of Type I meningococcus, namely Howes, was used in the present test, and the normal rabbit serum employed as diluent was well saturated with this coccus before use.

The dose of living coccus added to each tube was 0·00001 of a slope of Howes. The result of this experiment was as follows :

No.	*Serum.*	*Conc.*	*Complement.*	*Growth.*
1	Saturated		1 : 10	+
2	Normal rabbit		1 : 25	+
3	Serum (diluent)		1 : 50	≠
4	,,		1 : 106	≠
5	,,		1 : 200	≠
6	Normal horse	1 : 50	1 : 10	+
7	,,	,,	1 : 25	+
8	,,	,,	1 : 50	≠
9	,,	,,	1 : 100	≠
10	,,	,,	1 : 200	≠
11	Type I	1 : 50	1 : 10	(+)
12	No. 49	,,	1 : 25	(+)
13	,,	,,	1 : 50	+
14	,,	,,	1 : 100	≠
15	,,	,,	1 : 200	≠
16	Type II	1 : 50	1 : 10	+
17	No. 33	,,	1 : 25	+
18	,,	,,	1 : 50	≠
19	,,	,,	1 : 100	≠
20	,,	,,	1 : 200	≠
21	Diluent	..	..	≠
22	Normal horse	1 : 50	..	≠
23	Serum 49	1 : 50	..	≠
24	Serum 33	1 : 50	..	≠

It is seen that the complement at a dilution of 1 : 10 and 1 : 25 reduced the abundance of the meningococcus in all the four sera. This reduction was rather more marked with the Type I serum than with the rest ; and was also evident in the 1 : 50 dilution in that case. The lytic action of the Type I serum, however, was but feeble.

At this stage the present investigation had to be broken off owing to demobilization of the Central C.S.F. Laboratory. So far as the present observations go, however, the lytic effect on the meningococcus *in vitro* of the samples of serum examined cannot be said to have been by any means great, and in point of fact the intensity of their activity compared unfavourably with that of the fresh serum of a normal guinea-pig when diluted in the inactivated serum of a normal rabbit. It may be that the lysin content of the sera in question was really greater than appeared, and that the preservative in them interfered with the demonstration of their lytic action *in vitro* either by injuring the lysin, or by interfering with the action of complement, or in some other way. Had it been possible to continue the investigation, it was intended to have proceeded next to repeat these observations *in vivo* with a view to elucidating the matter further.

It should be mentioned that the Type I serum here examined for lysin was a sample of a batch that had proved clinically to possess an exceedingly high therapeutic value in Type I cases, since out of 66 of such cases treated with it or with similar serum, many of them severe, a few apparently moribund—no less than 59 (90 per cent.) recovered. That this striking therapeutic success was not dependent upon the lysin content of the serum is indicated by the present observations. A clue, however, to this potency lay in its possession of another antibody which will be considered in the following section.

In attempting to discern the part played by lysin in immunity to the meningococcus it must be borne in mind that, so far as available information goes, this antibody does not operate unless complement in active condition is also present. Exactly how far this dependence upon complement, or other activating factors, handicap lysin from freeing the living body of the meningococcus has not yet been determined. Important though the rôle of lysin may be in the defence of the *normal* animal against invasion by the meningococcus as indicated by experiments *in vitro* or *in vivo*, there is clearly a limit to its efficacy in man ; or invasion would never succeed. If from the present very incomplete study of lysin it is permissible to put forward a suggestion, I would venture to submit that as a constituent of therapeutic serum for administration to a patient in whom such invasion has already taken place, lysin may be of less benefit than an antibody that will neutralize the intracellular toxin of the particular meningococcus from the action of which the patient is suffering. During the temporary respite from intoxication resulting from the administration of such a serum, other factors in the defence, including lysin, opsonin, and in particular the patient's leucocytes, may have an opportunity of reducing the abundance of the invading meningococci that they would not otherwise obtain.

16. Antiendotoxin.

A. *Manner in which the importance of Antiendotoxin came to notice during the present outbreak.*

In course of the experimental study of the pathogenic action of the meningococcus described in a preceding section, it was found

that an important factor in the pathogenicity of this micro-organism consists of a powerful toxic agent or endotoxin which is contained within the body of the coccus, and is closely bound up with the bacterial protein. The toxic agent in question was found to withstand desiccation, boiling, and sometimes even exposure for a limited period to a temperature of 120° C. While soluble in water or saline, this toxic agent has not so far been extracted successfully from the coccus either by acetone, alcohol, or ether. The exact manner in which this toxic agent produces death in a susceptible animal has not yet been defined, but rabbits injected intravenously with an extract of the dried coccus containing it, succumb with symptoms of collapse and a rapid fall of temperature—symptoms similar to those observed by Flexner when studying the action of the living meningococcus on guinea-pigs. When injected intraperitoneally into mice, the dried meningococcus containing this toxic agent produces death in 24–48 hours. As a rule the mice become collapsed and more or less paralysed ; but exceptionally they may develop a certain amount of muscular spasm, and very rarely convulsions. Although attempts to demonstrate the presence of this toxic agent free in fluid cultures of the meningococcus or in the peritoneal fluid of guinea-pigs heavily injected with the living meningococcus have so far been unsuccessful, it is undoubtedly liberated when the coccus is lysed either by an external lysin or by the autolytic enzyme contained by the coccus itself. A probable illustration of its mode of action while still retained by the coccus is afforded by the intracellular reductase which, while similarly contained within the body of the meningococcus and not yet detected in washings of it, nevertheless succeeds in producing an intense reduction in the immediate environment of the meningococcus as demonstrated by the bleaching of methylene blue.

The great importance of the means of defence against this intracellular poison of the meningococcus came to light in the following manner. In the first year of the outbreak the antimeningococcus serum available failed to reduce the gross mortality in military cases to below 48 per cent. During the next recrudescence of cerebrospinal fever, however, in spite of the gross mortality keeping up to near its previous level, a consignment of serum in use in the London District was found to reduce the mortality there to 18·5 per cent., and apart from its effect on the mortality, this serum was found to produce a very striking improvement in the clinical condition of the patients shortly after its administration. On comparing this successful serum with samples of antimeningococcus serum that had been tried clinically and found to possess far less therapeutic value, the superiority of the former was found to lie neither in its agglutinin content, nor in its yield of complement-fixing antibodies, nor in its opsonin content, nor apparently in its ability to protect mice against the living coccus as tested by the procedure of Hitchens and Robinson. The sole difference that could be detected between the potent serum and the others lay in the fact that 0·5 c.c. of this therapeutically successful serum, when mixed with 1 M.L.D. of the dried bodies of either Type I or Type II Meningococcus and injected into the peritoneal cavity of the mouse, was found to preserve this animal

from death, whereas the other specimens of antimeningococcus serum all of which had proved to be of inferior clinical value without exception failed to do so.

While the qualities of the clinically successful serum were being investigated in this way the stock of good serum became exhausted, and the mortality among cases of cerebrospinal fever in the London District under treatment with the only antimeningococcus serum available, receded to the neighbourhood of 50 per cent. Directly the importance of antiendotoxin as an index to the therapeutic potency of the serum was discovered, therefore, samples were obtained of all the batches of antimeningococcus serum in store, and the capacity of each examined in the same way with regard to its ability to neutralize the endotoxins of the dried meningococcus of Types I or II in the peritoneal cavity of the mouse. As a result, a pooled serum was selected of which 0·5 c.c. was found to neutralize 1 M.L.D. of dried specimens of each of these two commonest types of the meningococcus respectively. On trial clinically in the London District and elsewhere this serum was now found to be quite as successful as the best serum used previously, especially in cases infected by Type I for which it had been found in the laboratory tests to contain more antiendotoxin than for Type II.

A few months before the close relationship between the clinical potency and antiendotoxic capacity of antimeningococcus serum had come to notice in this way, the Medical Research Committee had at my request given us facilities for an attempt to prepare antimeningococcus serum, and had provided the valuable services of a member of their staff, Dr. Stanley Griffith of the Field Laboratories, Cambridge, for the purpose of preparing the horses. The War Office also gave all assistance within their power; without which timely help the work could not have been carried on. Dr. Griffith prepared the horses by intravenous injection of the living coccus; his general mode of dosage being founded on the intensive method elaborated a short time before by Amoss and Woollstein of the Rockefeller Institute. A special feature, however, of the present procedure was that each horse was only prepared against meningococci of a single serological type as defined by the agglutination and absorption test. Before being put into supply, samples of serum from these horses were sent to the Central C. S. F. Laboratory for examination. The samples of serum submitted by Dr. Griffith have been found as a rule to possess an agglutinin titre for the type coccus of about 1 : 4,000. With this agglutinin value the opsonin content of the serum for the coccus of the homologous type was found by Major Tulloch to be apparently high, and the complement-fixing antibodies were found by Major Bell to be as a rule somewhat above the agglutinin reading (p. 67). In view of the clinical experience referred to, however, this serum was not passed for therapeutic use until 0·5 c.c. of it gave some evidence of neutralizing in the peritoneal cavity of the mouse the endotoxin of a dried coccus of the corresponding type. This antiendotoxic standard has caused the chief difficulty; and up to the present, in spite of every effort, the antiendotoxic capacity of the Type II serum for Type II meningococcus when determined in the manner stated has not on the whole

been nearly so good as the neutralizing capacity of the Type I serum for the representative cocci of that type selected for the purpose of standardization.

Before proceeding to give details of the clinical results obtained with this serum, it is desirable to refer briefly to the literature on the subject of antiendotoxin ; to describe the procedure employed when testing the serum ; and also to relate some experimental observations on the generation of antiendotoxin, and on the subject of its valency.

B. *The Present state of knowledge concerning Meningococcus Antiendotoxin.*

Reference to the literature shows that comparatively little is known at present regarding antiendotoxin, and that as is more or less inevitable under such circumstances the subject is involved in controversy. The present position would appear to be as follows. It is agreed that the majority of the pathogenic bacteria fail to secrete a soluble toxin, and that the bodies of these bacteria, or extracts of them, are toxic to animals ; there is, however, a dispute as to the nature of the substance which produces this toxic effect. On the one side are those who think with Pfeiffer that the toxicity is due to a specific intracellular toxin or endotoxin contained by the bacterial bodies, while their opponents declare with Vaughan and others that the toxic effects are not specific, and are due solely to split products derived from the bacterial protein. The crucial test of course is the production of antiendotoxin, and the view at present in favour appears to be that so-called endotoxin is distinguished from a true bacterial toxin such as that produced by the diphtheria or tetanus bacillus by the very fact that when injected into the animal body it fails to excite the production of antiendotoxin (cf. Zinsser).

On general grounds, however, it would appear unlikely that such impotence obtains on the part of the body to defend itself on the usual specific lines against what are admittedly the most active poisons of the majority of pathogenic bacteria. As the production of a specific antibody is far more difficult to demonstrate in the case of endotoxin which is tenaciously retained by the bacterial cell than in the case of a toxin which is readily soluble and diffusable, it seems rash to conclude that antiendotoxin is not formed merely because of some negative animal experiments. In point of fact, however, the positive formation of specific antiendotoxins has already been demonstrated. It is now some 12–14 years since Besredka in a brilliant piece of work first elaborated his well-known technique for extracting endotoxin from bacterial bodies, and then by making some careful tests of the power of the serum of horses under immunization to neutralize such endotoxin, definitely established the fact that the endotoxins of the micro-organisms of plague, typhoid, and bacillary dysentery are specific since they excite the production of specific antiendotoxins. It is noteworthy that Besredka found that the best way to prepare serum containing typhoid antiendotoxin was to inject the animal intravenously not with endotoxin, but with the

living bacterial bodies. Dopter confirms this in the case of dysentery and also in that of meningococcus, where he found that the serum of a horse prepared solely by the intravenous injection of the living bodies of meningococci, neutralized more endotoxin in an autolysate than did the serum of a horse prepared with the autolysate. It is interesting to note that Dopter mentioned incidentally in this communication that the antimeningococcus serum which possessed the higher yield of antiendotoxin was found to be superior to the other when tried clinically in cases of cerebrospinal fever.

In addition to Dopter, Jochmann, Kolle and Wasserman, Flexner, and Kraus and Doerr, have all observed the positive capacity of antimeningococcus serum to neutralize the endotoxin of the meningococcus set free by autolysis. Kraus and Doerr even went so far as to advocate the antiendotoxic method to the exclusion of all others for titrating the therapeutic value of antimeningococcus serum. Their procedure was to leave varying amounts of an autolysate of which the toxicity was known in contact with 1 c.c. of the serum for half an hour at 37° C., and to then inject the mixtures into the peritoneal cavities of young guinea-pigs of about 150–180 grm. weight. They found that 1 c.c. of antimeningococcus serum could neutralize from 2–10 M.L.D. of the endotoxin. Kolle and Wassermann thought that a serum of which 1 c.c. neutralized 5 M.L.D. was suitable for therapeutic use. The first difficulty, however, of this mode of standardizing antimeningococcus serum lies as Dopter has pointed out in the irregular toxicity of the autolysate of which sometimes 0·1 c.c. might produce death, while the animals receiving 0·3 c.c. survived. In order to get greater constancy in the lethal power of the autolysate, Kraus and Doerr allowed the cocci to macerate in N/10 NaOH instead of distilled water, but though this was an improvement the results were still found by Krumbein and Diehl and by Dopter to be irregular. A second objection raised by Dopter to this method of standardizing antimeningococcus serum lies in the probability that although a part of the microbial poison passes into solution in the process of autolysis, some of it remains behind in the bacterial bodies. A third objection mentioned by Dopter on the authority of Krumbein and Diehl is that the results vary according to the origin of the extract employed, since all meningococci do not give a toxin equally neutralizable by the same serum, and inversely the same extract is not always neutralized by serums of different origin. The conclusion arrived at by Dopter from these considerations is that no method of accurately estimating the antitoxic power of antimeningococcus serum exists, and even if it did, he does not agree with Kraus and Doerr that the antitoxic power of the serum is sufficient to serve as an absolute criterion of its curative value ; since the infection has to be dealt with as well as the intoxication. In short, according to Dopter, a method of accurately titrating the therapeutic value of antimeningococcus serum does not exist.

From this brief survey of the literature, therefore, it would appear

1. That the formation in the animal body of a specific antibody capable of neutralizing the endotoxin of the meningococcus contained

by an autolysate of that micro-organism is established beyond all question.

2. That there may be some difficulty in accurately standardizing serum by its power to neutralize the endotoxin in an autolysate of meningococcus.

3. From the observations of Krumbein and Diehl it is probable that the valency of meningococcus antiendotoxin is limited.

Against these points must be placed the following :

1. Practical experience during the outbreak had forcibly brought to light the great importance of the presence in antimeningococcus serum of antibodies capable of neutralizing the endotoxins of the two commonest types of the meningococcus ; the heavy mortality in certain stages of the outbreak having apparently been directly associated with a deficiency of the serum in such antiendotoxic properties.

2. That by using the dried coccus we were in possession of a method of obtaining endotoxin from the meningococcus not hitherto employed, and free of some of the criticisms of Dopter ; and by applying this test to samples of serum, a serum had already been selected that proved on clinical trial to be a striking therapeutic success.

3. That as defined by the agglutination and absorption tests, the types of meningococcus at work in our outbreak, as well as their individual frequency, were known ; and since monotypical sera for therapeutic use were already in course of preparation against these types, there was prospect that the valency of the various type anti-endotoxins could be determined not only by animal experiment, but also by the result of treatment of patients suffering from cerebro-spinal fever.

C. *Mode of testing Serum for Meningococcus Antiendotoxin.*

(i) *Preparation of the dried Coccus.*—A meningococcus is selected of good virulence, known serological type, and representative of current strains. After being submitted to daily subculture on trypagar slopes, trypagar plates are inoculated with the coccus and incubated for 18 hours at 37° C. A few cubic centimetres of saline are then poured into each plate, the growth detached by raking it off with an iron wire, and the suspension poured into a sterile test tube. After a film has been made of each suspension, stained by Gram and examined microscopically to exclude contaminations, the suspensions are mixed and centrifuged at high speed till the cocci are deposited. The majority of the supernatant fluid is then thrown away and with a pipette actuated by a rubber teat the cocci are transferred to a sterile glass dish which is supported over sulphuric acid in a glass desiccator. A few drops of ether are added in order to prevent growth of contaminations, the desiccator is exhausted of air by a vacuum pump, closed and placed in the incubator at 37° C. over night. Next morning the dish is found to be covered by a dry deposit which is scraped off, powdered in a glass mortar, and after microscopical examination transferred to a small glass-stoppered bottle. This process is repeated until sufficient of the dried coccus has been obtained. It has been found that 100 plates yield about 1·8 grm. of the dried coccus, and that 1 mg. of the dried coccus represents the dried deposit of about 7,000 million cocci.

From present observations it would seem that the dried coccus when kept in a glass-stoppered bottle in a cool dry place in the dark preserves its toxicity indefinitely.

(ii) *Preparation of the Endotoxin for Serum Tests.*—Suspension in water. In the first experiments, 0·1 grm. of the dried coccus was ground up slowly in an agate mortar, distilled water being gradually added to 5 c.c. This suspension can be used for standardizing serum and can be preserved by adding a few drops of ether to it. The objection to this method of suspending the coccus is that the particles keep depositing. It was next found that the supernatant fluid from such a suspension, which is of a slightly opaque and greenish tint, is toxic to mice from the endotoxin which it has dissolved out of the dried cocci, and for a time this fluid was used. The results, however, were not regular enough to give satisfaction, and after a short time this method was abandoned in favour of the following, which has been used in the vast majority of the tests.

Suspension in N/40 NaOH. The steps by which this procedure was arrived at have been described in a previous section (pp. 47, 48). 0·05 grm. of the dried coccus is ground up in an agate mortar for a few minutes in 1·25 c.c. of distilled water; 1·25 c.c. of N/20 NaOH is then added and the grinding continued for about a minute. It will be noticed that when the alkali is added the particles of the dried cocci go up more or less into solution. Where larger amounts of endotoxin are required successive amounts of the dried coccus can be treated in the same way and mixed. Measured amounts of the fluid are at once transferred to watch-glasses by means of a 1 c.c. pipette graduated in decimal divisions.

(iii) *Mode of testing the Serum.* A series of watch-glasses are put out each in a glass capsule. The M.L.D. of the toxin—generally 0·1 or 0·15 c.c. of the fluid just mentioned—is measured into each. To each watch-glass is then added 0·5 c.c. of serum, using first normal serum, and then the various sera of test. The test is made in duplicate, when possible, so as to exclude the idiosyncrasies of individual mice. After adding the serum the watch-glasses are placed in the incubator for 30 minutes and the contents of each of them are then injected intraperitoneally into mice of between 12 and 20 grm. weight. These mice are kept in special mouse stables designed by Major Hine, and are kept under observation for 2–3 days.[1] The heart's blood of those that die is found on cultivation to be sterile. The following experiment (see next page) shows the result of a test of five samples of antimeningococcus serum against the endotoxins of two representative strains Type I and Type II meningococcus respectively:

It will be observed that the death of both the control mice injected with the endotoxin mixed with normal serum proves that a fatal dose of endotoxin was here used. Two of the sera under examination neutralized the Type I endotoxin and three did not. All of the five sera had some neutralizing action on the Type II endotoxin, but only three of them saved both mice. One of the samples,

[1] As the endotoxin produces a fall of temperature, it is essential to keep the mice after injection in a warm place, or they may all die. For this reason the wooden 'stables' designed by Major Hine are preferable to metal cages. A certain amount of skill is required in applying this test which is only to be acquired by experience.

namely serum C., protected the mice from both endotoxins. This serum, therefore, possessed sufficient antiendotoxin in 0·5 c.c. to neutralize the endotoxin of specimens of both the commonent types of the meningococcus. On the strength of this test the serum in question was recommended for clinical trial. It proved to be a great success.

Serum.	*Amount.*	*Type I Endotoxin.*	*Result.*	*Type II Endotoxin.*	*Result.*
Normal	0·5	0·2	+	0·2	+
,,	,,	,,	+	,,	+
A	0·5	0·2	+	0·2	+
	,,	,,	+	,,	..
B	0·5	0·2	..	0·2	+
	,,	,,	..	,,	..
C	0·5	0·2	..	0·2	..
	,,	,,	..	,,	..
D	0·5	0·2	+	0·2	..
	,,	,,	+	,,	..
E	0·5	0·2	+	0·2	..
	,,	,,	+	,,	..

\+ = Mouse died.
.. = Mouse recovered

General Remarks.—Occasionally mice injected with the mixture of normal serum and endotoxin do not both die. This is more marked with some specimens of normal serum than with others and suggests that normal serum may occasionally contain a small amount of antiendotoxin. Besredka noticed the same thing when testing normal serum against the endotoxins of plague and typhoid. Before the alkali addition was made to the dried cocci it was sometimes found that both control mice though severely ill, might recover. In that case the only index to the value of the serum was the question as to its preserving mice from illness. It is a curious fact that at certain stages in the immunization of an animal its serum may exhibit less capacity for neutralizing meningococcus endotoxin than normal serum. The explanation is not yet clear, but possibly it is due either to complete temporary suppression of antiendotoxin, or to an increase in the lytic action of the serum producing the liberation of more endotoxin from the dried cocci. It is advisable to avoid using much more than 1 M.L.D. of the endotoxin, since multiples of it may not produce death. Dopter has called attention to this phenomenon in his article referred to above. The explanation is unknown. It does not always occur; but it is sufficiently frequent to make it inadvisable to use more than 1 M.L.D. of endotoxin. Something of the same kind seems to have been observed occasionally with snake venom. In practice, trouble due to this idiosyncrasy of the endotoxin is eliminated by using 1 M.L.D. The test is made quantitative not by increasing the toxin, but by diluting the serum.

The greatest number of tests have been made against the endotoxins of representative specimens of Types I or II, but monovalent sera of all four types have been tested against the homologous endotoxin. So far over 1,000 samples of serum of one sort or another have

been tested for antiendotoxin in this way. The great majority of them have shown but little evidence of antiendotoxin; but the sera that have passed with honour and have been tried clinically have produced results that more than justify the test as a valuable means of determining the potency of antimeningococcus serum.

D. *Experimental Observations on the production of Meningococcus Antiendotoxin.*

Shortly after the importance of antiendotoxin had come to light in the manner described, four series of observations were carried out on rabbits for the purpose of obtaining some information as to the conditions under which meningococcus antiendotoxin is produced. Young animals of between 1,000 and 2,000 grm. weight were used, and all of the injections were made intravenously. Comparison was made of the relative antigenic value of a number of different preparations of the same meningococcus. From time to time a sample of blood was taken from each rabbit, and the capacity determined of 0·5 c.c. of the serum for neutralizing 1 M.L.D. of the endotoxin of the homologous coccus. In the later tests when 0·5 c.c. of the serum was found to be successful in this respect, smaller amounts of it were also examined in the same way.

Series i.

Eleven rabbits were prepared with Type I meningococcus, strain Howes, as follows: two with the raw coccus killed by suspending it in saline containing 0·5 per cent. of phenol; two with the same suspension sensitized by leaving it in contact with immune serum all night, then removing the serum and resuspending the agglutinated cocci in phenol saline. Two further rabbits were prepared with the suspension of the coccus in phenol saline after leaving it for three days at 37° C. in order to promote autolysis. The five remaining rabbits were all injected with an aqueous extract made by grinding up 0·1 grm. of the dried coccus and slowly adding 5 c.c. of water during the grinding. A few drops of ether were then added to the suspension, which was allowed to stand at room temperature over night in order to deposit the cocci. Next morning the supernatant fluid was pipetted off, phenolated, and used for preparing the five rabbits. The injections were made at intervals of 4–5 days. The initial dose in case of the suspensions represented an amount containing approximately 500 million cocci; and in case of the watery extract 0·05 c.c., an amount representing the extract from 1 mg. of the dried coccus.

The result of the present experiment is summarized in the table on p. 102.

It will be observed that of the eleven rabbits no less than six succumbed in course of preparation against this Type I meningococcus. Of the various antigens compared, the raw coccus was here a complete failure since neither rabbit gave any evidence of antiendotoxin and both died. Both rabbits injected with the sensitized coccus gave promising results in the first test, and the rabbit that survived continued to show antiendotoxin in the later tests. The autolysed coccus gave good results in the first two tests, and then

both rabbits became negative. Of the five rabbits receiving the extract three died, and only two gave evidence of antiendotoxin.

Antigen.	*Five days after fourth dose.*	*Five days after sixth dose.*	*Nine days after ninth dose.*
Raw coccus	..	..	D
Do.	..	..	D
Sensitized coccus	+	D	
Do.	+	+	+
Autolysed coccus	+	+	..
Do.	+	+	..
Extract of dried coccus	+	+	D
Do.	..	..	+
Do.	..	..	..
Do.	..	..	D
Do.	..	D	..

+ = Antiendotoxin present.
D = Rabbit dead.

Series ii.

While the preceding observations were in progress, another set was started in which four rabbits were prepared with equal doses of the same coccus (Type I Howes) as follows :

1. Coccus raw.
2. Coccus sensitized.
3. Coccus dried.
4. Coccus dried and then sensitized.

All these four antigens were prepared from the same suspension. The doses administered to the rabbits were 500 million, 1,000 million, 2,000 million, and 4,000 million cocci at intervals of five days. Four days after the last dose the serum of each rabbit was tested for antiendotoxin with the result that the rabbits prepared with the raw coccus and with the dried coccus after sensitization were positive, the other two negative. In order to see the effect of increasing the dose, all of the rabbits now received at weekly intervals a dose of 10,000 million, 20,000 million, and 25,000 million, and six days after the last dose their serum was once more examined for antiendotoxin with the result that all were now found to be negative.

While they failed to settle the question as to which is the best antigen to employ, therefore, the two preceding series of experiments brought out an important point, namely, that in order to obtain the presence of antiendotoxin in the serum of an animal under immunization, it is very necessary not to raise the dose too high. In addition to seriously endangering the life of the animal, super-immunization, so far from increasing the yield of antiendotoxin, may cause it to disappear.

Series iii.

At the time that Series i. was started a similar group of eleven rabbits was prepared in the same way with Type II meningococcus, strain Morgan. These rabbits received doses of the same size as those given to rabbits in Series i. at intervals slightly longer, and the dose was raised in much the same way. The first dose was 500 millions and the last 10,000 millions. In contrast to what happened in Series i., none of the present rabbits died during immunization, an

experience that confirms a previous observation, namely, that Type I is apt to be more toxic than II to laboratory animals. No examination was made of the antitoxic value of the serum of these rabbits until they had been under preparation for a period of three months during which they received nine injections. Ten days after the last injection a sample of blood was taken from each animal, and the serum tested with regard to its capacity to neutralize the endotoxin of the homologous coccus, and when 0·5 c.c. of the serum was positive smaller amounts were tested in the same way. The results are shown in the following table :

	Serum.		
Antigen.	*0·5*	*0·25*	*0·1*
Raw coccus	..		
Do.	+	+	+
Sensitized coccus	+	..	
Do.	+	..	
Autolysed coccus	..		
Do.	..		
Dried coccus	+	+	+
Do.	+	+	..
Do.	+	+	..
Do.	+	+	..
Do.	..		

+ = Antiendotoxin present.

On comparing these results with those obtained in Series i. it would appear that from the point of view of the production of antiendotoxin in the rabbit, the Morgan coccus is less toxic and a better antigen than Howes. Here one of the two rabbits prepared with the raw coccus was a failure,the other a great success, since so small an amount as 0·1 c.c. of its serum neutralized 1 M.L.D. of the endotoxin. Both of the rabbits prepared with the sensitized coccus were moderately successful : both of those prepared with the autolysed coccus failed. Of the five rabbits prepared with the dried coccus no less than four neutralized 1 M.L.D. of the endotoxin with 0·25 c.c. of their serum, while one of them succeeded also with 0·1 c.c. In the present experiment, therefore, the dried coccus and perhaps the sensitized coccus appeared to be the most consistently successful antigens.

Series iv.

The next step was to resume comparison of the antigenic value of various forms of the Type I coccus, strain Howes, but with special precautions against overdosage. With this end in view the following procedure was adopted :

(1) As before, a moderate initial dose consisting of 500 million cocci of the suspension was given. In case of the dried coccus the whole suspension was used, and the first dose was 0·05 c.c. containing 1 mg. of the dried coccus.
(2) Before raising the dose, the preceding dose was repeated.
(3) The dose was raised very gently, e. g. in case of the raw coccus the dosage was 500, 600, 700, 800, 900, and 1,000 millions progressively. In case of the dried coccus the amount given was 0·05, 0·06, 0·07, 0·08, 0·09, and 0·1 c.c. of the suspension.

(4) Lastly, in order to see if even this dosage was overtaxing the powers of the animal, a duplicate series of rabbits was used in which the initial dose was maintained throughout, and not raised at all.

As before, the antigens compared were the raw coccus, the sensitized coccus, and the dried coccus. As an extra, comparison was made of the antigenic value of the raw and dried coccus respectively after their endotoxin had been inactivated by leaving the coccus in contact with N/1 NaOH overnight at 37° C., the alkalinity being then reduced to N/40.

The rabbits in the present series were injected every 48 hours, and each received altogether ten doses. Their serum was tested against the endotoxin of the homologous coccus on the following occasions: Two days after the first dose; one day after the fifth dose; one day, three days, and five days after the tenth dose. The specimen of endotoxin against which the samples of serum were tested was specially selected because of the uniformity with which it produced death in a number of control mice used at this time for testing the serum of horses in process of preparation against Type I.

The result of the present observations is summarized in the following tables where the amount of serum used is stated in each test.

DOSE STATIONARY.

	Serum.					
	One day after first dose.	*One day after fifth dose.*	*One day after tenth dose.*			*Two days after tenth dose.*
Antigen.	*0·5*	*0·5*	*0·5*	*0·25*	*0·1*	*0·5*
Raw coccus	..	+	..			
Sensitized coccus	..	..	..			
Dried coccus	..	+	+	+	..	..
Raw coccus N/40 NaOH	..	..	..			
Dry coccus do.	..	..	..			

DOSE RAISED.

	Serum								
	Two days after first dose.	*One day after fifth dose.*	*One day after tenth dose.*			*Three days after tenth dose.*			*Five days after tenth dose.*
Antigen.	*0·5*	*0·5*	*0·5*	*0·25*	*0·1*	*0·5*	*0·25*	*0·1*	*0·5*
Raw coccus	..	..	..			..			
Sensitized coccus	..	+	+	..		+	..		..
Dried, do.	..	+	+	+	+	+	..		..
Raw coccus N/40 NaOH	..	..	..			..			
Dried coccus, do	..	..	..			..			

+ = Antiendotoxin present.

It is seen that the serum of all the rabbits was negative at first.

1. With regard to the series in which the dose was stationary the raw coccus produced some antiendotoxin after the fifth dose, but it vanished later. The dried coccus was the most successful in this

series as might have been anticipated, because the dose was relatively higher than in the case of the other antigens.

2. As regards the series in which the dose was raised, the sensitized coccus was more successful than in the first series. The most successful antigen, however, was the dried coccus which 24 hours after the tenth dose had evoked the production of sufficient antiendotoxin to neutralize 1 M.L.D. of endotoxin with 0·1 c.c. of the rabbit's serum. It is interesting to note, however, that the serum of the rabbit that received the stationary dose of this same antigen neutralized the same amount of endotoxin with 0·25 c.c. of its serum on this day. Twenty-four hours later the antiendotoxin had gone from the serum of the rabbit that had received the stationary dose, but was still present in the serum of the other, though in diminished quantity, and two days later it was gone. The cocci of which the endotoxin had been destroyed by NaOH gave negative results.

Conclusions.—These observations were held to indicate :

1. That antiendotoxin is elaborated by the rabbit to both the commonest types of the meningococcus.
2. That in order to obtain antiendotoxin, especially in the case of Type I meningococcus, it is absolutely essential to avoid overdosage.
3. That of the antigens compared, the most promising appeared to be the dried coccus, and the sensitized raw coccus.

E. *The Valency of Meningococcus Antiendotoxin.*

The question now arises as to the range of the activity of meningococcus antiendotoxin. Has antiendotoxin to a given strain of the meningococcus any capacity to neutralize the endotoxins of meningococci of other serological types ? Does it neutralize the endotoxins of all specimens of the same type as defined by the agglutination test ? It is clear that some light on these matters may be expected from the result of the clinical application of the monotypical sera in the treatment of cases of cerebrospinal fever, and the available information under this heading will be considered later. With regard to the experimental investigation of the valency of antiendotoxin, it is to be regretted that owing to the pressure of other work it was not practicable to go into this matter at the time when the experimental observations just related on the production of meningococcus antiendotoxin by the rabbit were in progress. Later on the difficulty of getting therapeutic serum that strongly neutralized endotoxin, and the clinical demand for any of such serum as was available considerably delayed the investigation of valency. Before the Central C.S.F. Laboratory was demobilized, however, a number of cross tests were carried out with results that will now be related.

(1) Investigation of the capacity of two specimens of serum containing antiendotoxin for a specimen of Type I meningococcus to neutralize the endotoxins of meningococci belonging to Types II, III, and IV.

Particulars of these tests are shown in the following tables :

(*a*) v. Type I endotoxin.

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal	0·5	Type I, Smith	0·1	+
„	„	„	0·2	+
Type I, 44a . . .	„	„	0·1	..
„ . . .	„	„	0·2	..
Type I, 49a . . .	„	„	0·1	..
„ . . .	„	„	0·2	..

+ = Mouse died.

Both these specimens of Type I serum, therefore, neutralized the endotoxin of the Smith coccus.

(*b*) v. Type II endotoxin.

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal	0·5	Type II, Morgan	0·1	..
„	„	„	0·2	+
Type I, 44a . . .	„	„	0·1	..
„ . . .	„	„	0·2	+
Type I, 49a . . .	„	„	0·1	..
„ . . .	„	„	0·2	+

+ = Mouse died.

The two specimens of Type I serum, therefore, failed to neutralize the Morgan endotoxin.

(*c*) v. Type III endotoxin.

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal	0·5	Type III, Chase	0·1	..
„	„	„	0·2	+
Type I, 44a . . .	„	„	0·1	+
„ . . .	„	„	0·2	+
Type I, 49a . . .	„	„	0·1	+
„ . . .	„	„	0·2	+

+ = Mouse died.

The two sera were apparently less protective against the endotoxin of Chase than normal horse serum.

(*d*) v. Type IV endotoxin.

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal	0·5	Type IV, Hicks	0·1	..
„	„	„	0·2	+
Type I, 44a . . .	„	„	0·1	+
„ . . .	„	„	0·2	+
Type I, 49a . . .	„	„	0·1	..
„ . . .	„	„	0·2	+

+ = Mouse died.

The two sera also failed to neutralize the endotoxin of Hicks.

Confirmatory test against Type I endotoxin. As these two samples of Type I serum failed to neutralize the endotoxin of meningococci of the three other types they were once more put up against the endotoxin of the Type I coccus.

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal	0·5	Type I, Smith	0·15	+
„	„	„	0·15	+
Type I, 44a . . .	„	„	0·15	..
„ . . .	„	„	0·15	..
Type I, 49a . . .	„	„	0·15	..
„ . . .	„	„	0·15	..

Clearly, then, there is no doubt as to the capacity of the two samples of serum to neutralize the endotoxin of the Smith coccus.

(2) Examination of the capacity of the same two samples of Type I serum to neutralize the endotoxin of six individual specimens of Type I meningococcus.

All of these meningococci had been found by the agglutination and absorption tests to be examples of Type I. The result of the present test which was carried out in the same manner as the preceding ones is summarized in the following table :

Serum.	*Amount.*	*Endotoxin amount.*	*Specimens of Type I Meningococcus.*					
			Smith.	*Cooper.*	*M. 107*	*Mrs. F.*	*Pay.*	*Wild-Smith.*
Normal	0·5	0·1	+	+	..	..	..	+
,,	,,	0·2	+	+	+	+	..	+
Type I, 44a	,,	0·1	..	..	+	..	+	..
,,	,,	0·2	..	+	+	+	..	+
Type I, 49a	,,	0·1	..	+	..	+	+	..
,,	,,	0·2	..	..	+	+	..	+
Inference			Good protection.	Some protection.	No protection.	No protection.	No protection.	Some protection.

It would appear that while both the specimens of Type I serum gave some evidence of neutralizing the endotoxin of three of these Type I strains, they exhibited no such tendency in the presence of three further strains indistinguishable from them by the agglutination and absorption tests.

(3) Examination of the capacity of Type II serum to neutralize the endotoxins of two specimens of Type II meningococcus.

It has been pointed out that when investigated by the agglutination and absorption tests the meningococci included in Type II were found to be less uniform than in the case of Type I, and that in course of his serological study of this group Major Tulloch found that a number of sub-groups of Type II could be distinguished. By using a sufficiently representative specimen of Type II, however, it was found that a serum could be prepared that succeeded in identifying practically all members of the group by the agglutination and absorption tests.

Two of such specially selected and representative specimens of Type II that had been employed for making agglutinating serum were the Morgan coccus and later the Lindenbaum coccus. According to the agglutination and absorption tests these cocci appeared to be identical. It was of interest, therefore, to see if their endotoxins were identical or not. Both cocci had been recovered from rapidly fatal cases of cerebrospinal fever, and as in each of them a quantity of the coccus had been dried and stored shortly after isolation from the patient, any alteration due to subculture could be excluded when comparing their respective endotoxins. The result of comparison of the endotoxins of these two representatives of Type II against the same specimen of Type II serum is seen in the following tables :

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal . . .	. 0·5	Type II, Morgan	0·1	+
,, . .	. ,,	,,	0·2	+
Horse 27, 28/7	. . ,,	,,	0·1	+
,,	. . ,,	,,	0·2	+

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal . . .	. 0·5	Type II, Lind.	0·1	+
,, . .	. ,,	,,	0·2	+
Horse 27, 28/7	. . ,,	,,	0·1	..
,,	. . ,,	,,	0·2	..

\+ = Mouse died.

The sample of serum from Horse 27, therefore, neutralized the endotoxin of Lindenbaum, and not that of Morgan.

Two other specimens of Type II serum were also found to give a precisely similar result to the above. On the other hand, an older specimen of Type II serum was found to have more neutralizing action on the endotoxin of Morgan than on that of Lindenbaum. It is of interest to note that the last serum, although it agglutinated the Lindenbaum coccus up to well over 1 : 2,000, had been found to be without effect when used therapeutically in the treatment of cases of cerebrospinal fever occurring during the outbreak in which the Lindenbaum coccus was isolated.

Further experiments in the same way brought evidence to light of a third endotoxin in Type II.

These cross tests, therefore, indicate that the types of meningococcus as defined by the agglutination and absorption tests are further subdivided by investigation of the antiendotoxins of individual strains belonging to them. Thus it would seem from the present evidence that the endotoxin of the meningococcus is even more specific than its bacterial protein. When preparing monotypical serum for therapeutic use, therefore, it is advisable to use a plurality of strains of each type, the number being dependent on the diversity exhibited by the endotoxins of individual meningococci within each type, and by their relative frequency.

F. *Clinical Results with the Monotypical Sera.*

These are analysed by Major Hine on pages 176–188.

G. *Conclusions with regard to Antiendotoxin.*

The observations related in the present section have led to the belief that from the point of view of its therapeutic efficacy, antiendotoxin is the most important of all the individual antibodies identified so far in antimeningococcus serum. Unfortunately the study of antiendotoxin is at present a very arduous matter owing to the difficulty of extracting it from a micro-organism so minute, elusive, and complex as the meningococcus, as well as to the difficulty of preparing antiendotoxin. Progress, nevertheless, is possible owing to the stability of endotoxin, and to the elaborate resources of the immunizing mechanism of the body.

With an improved method of extracting endotoxin and obtaining it in solution, it should be possible to study the conditions favourable

to the production of antiendotoxin more closely, and also to define the valency of these antibodies more thoroughly than has been possible up to the present time.

The conception of cerebrospinal fever as a disease in which one of the crucial features is the struggle between the endotoxin of the infecting meningococcus and the capacity of the patient to generate antiendotoxin, has the advantage that it brings this disease into line with infections in which serotherapy has achieved some of its greatest successes. Owing to the limited diffusion of the endotoxin contained by the meningococcus, antiendotoxin in antimeningococcus serum applied at the site of infection should go considerably farther than does the antitoxin used in diphtherin where the infecting micro-organism is actively secreting a soluble toxin which is readily diffused throughout the body. This is the reason, perhaps, why a comparatively small amount of the *correct* antiendotoxin in anti-meningococcus serum appears to have such an excellent therapeutic effect in cerebrospinal fever. One of the chief hopes of progress in the specific therapy of this disease, therefore, would appear to lie in the preparation of serum more consistently rich than hitherto in specific antibodies capable of neutralizing the endotoxins of the commonest forms of the meningococcus.

17. The Protective Power of Antimeningococcus Serum.

In course of an experimental study described in a preceding section evidence was found that the pathogenic action of the meningo-coccus is dependent upon two different factors, namely, its intra-cellular toxin, and the ability of the coccus to multiply in the tissues of its host. For reasons described previously, chief attention has been paid to the endotoxin, and especially to the means of defence against it. Some preliminary observations, however, have also been made of the value of antimeningococcus serum for protecting against invasion by the living meningococcus ; and these it is now proposed to consider.

A plurality of observers including Bonhoff, Jochmann, Flexner, Ruppel, v. Lingelsheim, and recently Hitchens and Robinson, have found that the resistance of an animal to invasion by the meningo-coccus can be increased by giving it a prophylactic injection of suitable antimeningococcus serum. In some observations on this matter made by T. J. Horder and myself in 1908, we found this protective action to be wanting in the case of the particular specimens of antimeningococcus serum examined by us, and in no way com-parable to the protection conferred on the animal by prophylactic vaccination with the meningococcus. From a study of the literature, however, it would appear that the chief reason why examination of the protective value of antimeningococcus serum against the living meningococcus has not been carried out more extensively is owing to the unstable virulence of the meningococcus for laboratory animals. Apart, also, from this technical difficulty in applying the test in a satisfactory manner, it is open to question whether serum that succeeds prophylactically is necessarily the best for general therapeutic use. In this relation it may be repeated that

when compared with regard to their protective power against the living meningococcus, no salient difference could be detected between the serum that had been found to possess a high potency when administered to cases of cerebrospinal fever, and specimens of serum of far inferior clinical value.

In the procedure advocated by Hitchens and Robinson mice are injected intraperitoneally with 0·5 c.c. of the serum, and two hours later are again injected with falling amounts of the living coccus by the same route. This method has the advantage that fluctuation in the virulence of the coccus does not invalidate the test. Its chief disadvantage is that the mouse receives two intraperitoneal injections within a comparatively short space of time. For this reason, and also because during an attempt to define the end-point of a serum that definitely protected against the living meningococcus, it was found better to keep the dose of coccus constant and to dilute the serum, the proceeding of Jochmann was followed in the present tests. In this procedure the mouse is injected subcutaneously with a small amount of serum, and 24 hours later the animal receives intraperitoneally a minimal lethal dose of the living coccus. For measuring the latter Jochmann used the standard loop, but in the present experiments 0·5 c.c. was used of a freshly made suspension of a slope culture of the meningococcus in warm broth. As the coccus decreases in broth the injections should be made as soon as possible after adding the broth to the slope. The dose of coccus depends of course on the virulence of the particular strain employed, and must be carefully determined by repeated tests beforehand since the M.L.D. may vary in different strains from 1/40 to 1/5 or more of a slope. It is advisable to select a strain that shows constancy in its M.L.D. rather than to employ one that is perhaps more virulent, but less certain in killing power. In carrying out the test a control experiment is made with normal serum, and so many are the fallacies when working with the living coccus, that confirmatory tests are here more than ever necessary before the result can be accepted.

The point upon which information was sought in the present instance was the question as to whether an amount of serum that sufficed to neutralize a fatal dose of endotoxin of a given meningococcus necessarily succeeded in protecting against invasion by the same coccus.

The coccus employed was Type I, strain Howes. The result given by three specially selected samples of serum when tested against the endotoxin of Howes and against the living coccus respectively are seen in the following tables.

ANTIENDOTOXIC VALUES.

Serum.	*Amount.*	*Endotoxin.*	*Amount.*	*Result.*
Normal	0·5	Type I, Howes	0·1	+
,,	0·5	,,	0·2	+
Serum A	0·5	,,	0·1	..
,,	0·5	,,	0·2	..
Serum B	0·5	,,	0·1	+
,,	0·5	,,	0·2	+
Serum C	0·1	,,	0·1	..
,,	0·1	,,	0·2	..

+ = Mouse died.

ANTIMICROBIAL VALUES.

Serum.	*Amount.*	*Coccus.*	*Amount (slope.)*	*Result.*
Normal . . .	0·5	Type I, Howes	0·2	+
,, . . .	0·5	,,	,,	+
Serum A . .	0·5	,,	,,	..
,, . . .	0·5	,,	,,	..
Serum B . .	0·5	,,	,,	..
,, . . .	0·5	,,	,,	..
Serum C . .	0·1	,,	,,	+
,, . . .	0·1	,,	,,	+

+ = Mouse died.

While 0·5 c.c. of serum A both neutralized the endotoxin and protected against the living coccus, the same amount of serum B failed to neutralize the endotoxin, but protected against the living coccus. In the case of serum C, on the other hand, 0·1 c.c. neutralized the endotoxin, but failed to save the mouse against the living coccus. It would seem, therefore, that while a serum may possess both antitoxic and antimicrobial powers, these qualities do not necessarily coincide.

As it is possible and even probable that the antimicrobial power of antimeningococcus serum may be of special value in the treatment of fulminating or other cases suffering from meningococcal septicaemia, further study of this antimicrobial property of antimeningococcus serum is very desirable, particularly with a view to determining its valency, and also with a view to defining the particular antibodies with which this function is associated.

18. A study of Gram-negative Diplococci of the Nasopharynx resembling the Meningococcus.

By W. J. Tulloch, O.B.E., M.D., Brevet-Major R.A.M.C.
(Lecturer in Bacteriology at the University of St. Andrews).

The results which are dealt with in this communication were obtained in making an investigation of the relationship which Gram-negative diplococci of nasopharyngeal origin bear to meningococci of known pathogenic significance, and the research was undertaken with a view to testing the value of the agglutination reaction as a means of detecting carriers of the meningococcus.

A. *Introduction.*

It might be contended :

(I) that a certain number of meningococci when recently isolated from pathological material fail to agglutinate.

(II) that the agglutination of the meningococcus presents such difficulties of technique, and

(III) that the varieties of that organism are so numerous as to render invalid any attempt that might be made to differentiate, by the agglutination method, meningococci from other Gram-negative diplococci inhabiting the nasopharynx of man.

It is important that these arguments be carefully considered, for,

should they prove of practical importance, both the prophylaxis and serum therapeutics of epidemic cerebrospinal meningitis among troops would present almost insuperable difficulties.

That relatively few meningococci fail to agglutinate is shown by the fact that of a series of spinal strains of meningococci numbering 500, examined at the Central C.S.F. Laboratory during the past three years, only 8 failed to agglutinate in the presence of the four-type agglutinating sera. It has been the experience, moreover, of the workers at the Central C.S.F. Laboratory and the London District Laboratory, that meningococci of nasopharyngeal origin, when recently isolated, agglutinate more readily than do the spinal strains of the organism.

Further, in a paper published in the *Journal of the Royal Army Medical Corps* for July 1917, I showed that the large majority of Gram-negative diplococci of spinal origin submitted to scrutiny exhibited unequivocally the characters of one or other of the four serological types of the meningococcus.

Since the publication of that paper further evidence, based on the examination by the absorption method of over 200 specimens of spinal origin, has been obtained which shows that the experimental error is small (2 per cent.) when the agglutination test, performed according to the standard technique, is employed.

This practical experience proves that the contentions recapitulated in the preceding paragraph are not valid so far as the application of the agglutination test to spinal strains of the meningococcus is concerned.

It does not, however, dispose of these contentions in respect of cocci of nasopharyngeal origin.

In order to inquire further into this problem it was decided :

(*a*) To examine by means of the agglutination and absorption of agglutinin tests, a series of nasopharyngeal Gram-negative diplococci which failed to qualify as meningococci when tested by the standard agglutination technique.

(*b*) To immunize animals against representatives of these ' pharyngococci ' in order to determine their serological relationship to one another and to the meningococci.

B. *Material and its Source.*

The material on which the following observations are based was obtained from Captain Glover, Officer in Charge of the London District C.S.F. Laboratory, and I wish here to express my gratitude to him for supplying me with the cultures.

These cultures were derived from the nasopharyngeal secretion of non-contact (military) populations and I asked Captain Glover to hand over to me for the most part those cocci which failed, on preliminary agglutination, to qualify as meningococci ; but special care was taken also to include in the series a few of the nasopharyngeal cocci which Captain Glover regarded as true meningococci. In order that I might not be biassed in the interpretation of my own results I purposely had no access to the results of the preliminary routine examinations.

Of the cultures sent, 40 were picked out at random for complete investigation which comprised :

(*a*) Careful reisolation by plating.
(*b*) Microscopical examination.
(*c*) Ordinary cultural investigation.
(*d*) Examination of representative strains by culture in carbohydrate medium.
(*e*) Agglutination by the standard technique advised for serological identification of the meningococcus in contacts.
(*f*) The capacity of these micro-organisms to absorb the specific agglutinins from Type I, Type III, and Type IV sera, and also from two specimens of Type II serum by the technique described in a previous communication on the differentiation of meningococci (*R.A.M.C. Journal*, July 1917).

Two specimens of Type II serum should always be used in performing the absorption test owing to the complexity of the Type II group of meningococci.

(*g*) Preparation of ' pharyngococci-agglutinating ' sera which were used for investigating both the pharyngococci and known meningococci by the agglutination and absorption of agglutinins tests.

C. *Cultural Examination of the Nasopharyngeal Micro-organisms.*

When examining primary plates of nasopharyngeal swabbings, it is found that organisms which are liable to be mistaken for the meningococcus grow in colonies that have characters strongly resembling those of that micro-organism.

In making the examination, both the naked eye characters, and characters demonstrated when use is made of a hand lens multiplying 10–12 diameters, are noted. Those colonies which are worthy of complete investigation are characterized by the following points :

(*a*) They are about 1–3 mm. in diameter after 24 hours' incubation at 37° C.

(*b*) They have a sharp cut and, unless distorted by adjacent growth, perfectly circular outline.

(*c*) They are transparent.

(*d*) They are definitely lenticular in shape, resembling a drop of clear fluid on the surface of the medium.

(*e*) They are almost colourless when viewed by transmitted light.

(*f*) They are blue-grey in colour and exhibit a slight granularity at the centre of the colony with a clear non-granular peripheral zone when viewed against a dark background.

With the exception of (*a*) these characters are best demonstrated by use of the hand lens.

Such examination serves to exclude the majority of micro-organisms belonging to the flavus, M. pharyngeus siccus and catarrhalis groups, thus limiting the scope of the subsequent investigation by cultural and serological methods.

All the micro-organisms dealt with in the present communication exhibited the characters mentioned.

D. *Investigation of Representative Specimens of the Series by cultivating them in Carbohydrate Media.*

Before proceeding to examine the cultures by serological methods, 28 representatives of the series to be investigated were picked out at random for examination with regard to their growth in carbohydrate media.

Method.—Peptone water was prepared in the usual way and was carefully standardized to be just neutral to *a*-naphtholphthalein; the reaction was tested after the medium had been tinted with litmus and sterilized for 30 minutes at 100° C. on three successive days.

The carbohydrates used were:

(1) Glucose Puriss (Baird and Tatlock).
(2) Maltose prepared for me by Professor Irvine, F.R.S., Professor of Chemistry at the University of St. Andrews.
(3) Mannose also prepared by Professor Irvine.
(4) Saccharose (Baird and Tatlock).

I here wish to record my debt of gratitude to Professor Irvine for supplying me with mannose and maltose at the present time when really reliable specimens of these carbohydrates are almost unobtainable.

These reagents were made up in distilled water as 10 per cent. solutions, and were sterilized in this form on two successive days for 30 minutes at 100° C. On the second day, while still hot, they were added to the tubes of litmus peptone water in sufficient quantity to make the concentration of carbohydrate equal to 1 per cent. of the completed medium. The mixture of peptone water and carbohydrate was then finally sterilized by exposure to 100° C. for 15 minutes on two successive days.

Fresh sterile horse serum was now added to each tube to the extent of 10 per cent. of the total, and the tubes were then incubated for three days at 37° C., after which representative tubes of each series of sugars were cultivated to ensure that the tubes had not been contaminated.

The material used for inoculating the sugar tubes consisted of large loopfuls of a vigorous 20-hour growth on blood-smeared trypagar. The cultures in the sugar tubes were kept under observation from the second to the tenth day, and subcultures were made from them on the second and fifth days after inoculation to ensure that growth had occurred in those tubes which gave a negative result, and that when a positive reaction occurred it was due to the development of Gram-negative cocci and not to contaminating organisms.

Eight representative meningococci—two of each serological type—of spinal origin were also introduced as controls into the series.

Of the 28 cultures of pharyngococci thus tested
(*a*) Twenty-three gave the classical sugar reaction of the meningococcus;
(*b*) Four failed to ferment any of the sugars;
(*c*) One fermented glucose only.

Of the eight meningococci tested

(*a*) Six gave the classical reactions—acid in media containing glucose and maltose—in presence of the sugars.

(*b*) One—a Type I organism by agglutination and absorption—produced only very slight and transient acidity in presence of glucose and maltose, the former being demonstrated on the third, fourth, and fifth days, and the latter on the eighth, ninth, and tenth.

(*c*) One, a Type II organism by agglutination and absorption, failed to produce acid in presence of any of the sugars.

Only one of these 28 ' pharyngococci ', whose fermentation reactions were tested, proved on serological examination to qualify as a meningococcus. It gave the classical sugar changes and proved to be a Type I coccus.

These results show that the majority of micro-organisms of naso-pharyngeal origin which develop colonies resembling those of the meningococcus are found to give the fermentative reactions of that organism. These results suggest also that meningococci of spinal origin are not so constant in respect of their fermentative activities as is generally supposed.

E. *Examination of the* 40 *Gram-negative Cocci of Pharyngeal origin by the Agglutination and Absorption of Agglutinins Test.*

The whole series of 40 cocci was then tested by the standard agglutination method advised by the Central C.S.F. Laboratory for the detection of meningococcus carriers.

This test gave the following results :

(*a*) Twenty-three of the cocci failed to agglutinate in presence of any of the type agglutinating sera, or in presence of normal (rabbit) serum.

(*b*) Six agglutinated of a greater or less degree in presence of all the type sera and in presence of normal serum.

Of the 23 cocci which failed to agglutinate in presence of any serum, 16 had been included in the series tested by means of the sugars. These 16 comprised 13 which produced acid in presence of both glucose and maltose, one which fermented glucose only, and two which fermented none of the sugars.

All six which reacted with normal serum had been cultivated in the sugars ; five of them produced acid from glucose and maltose, and one fermented no sugar.

(*c*) Two of the cocci reacted feebly in presence of 1/100 dilution of Type II serum—end-point 1/400—but neither of these absorbed agglutinins from any of the type sera. One of them had been examined by growth in the sugar medium and produced acid from glucose and maltose.

(*d*) The remaining nine gave the reactions shown in the following table when tested by the standard agglutination technique. The sugar reactions, where these were investigated, are also indicated.

The reactions of the cocci shown in Table I present certain difficulties of interpretation. It has been the experience, however, of

the workers at the Central C.S.F. Laboratory in examining micro-organisms of nasopharyngeal origin that :

(1) Cocci which give a positive specific reaction, under standard conditions with one and with only one of the type agglutinating sera, end-point 1/400, to a dilution of 1/200, and do not agglutinate in presence of 1/50 normal serum, may be definitely classed as meningococci.

(2) Cocci, which react specifically in presence of 1/100 dilutions of any one of the type sera but react in presence of no higher dilution, are probably not meningococci. They should, however, be carefully scrutinized and a second examination should be made of men carrying such organisms.

(3) In interpreting results obtained with cocci which react in the presence of two of the type sera to a dilution of 1/200, or more, with each of these, and do not flocculate in presence of 1/50 normal serum, the following points are to be noted.

(*a*) There is a marked affinity between many meningococci of groups I and III, and between some of groups II and IV. Therefore micro-organisms which react with I serum and III serum, or with II serum and IV serum to a dilution of 1/200 or more, are to be definitely regarded as meningococci. In most instances such cocci can be placed as I or as III ; as II or as IV by determining the actual end-point of the reaction, in which case a positive result will usually be obtained with a higher dilution of the true type serum than of the other.

(*b*) When the coccus under examination reacts to 1/200 dilution, with two other of the Type sera, e. g. I and II or II and III and is negative with 1/50 normal serum, one regards the micro-organism as definitely a meningococcus if the reaction takes place with one of the sera to full titre, i. e. complete agglutination in presence of 1/400 dilution of the serum under standard conditions.

If the coccus reacts only to 1/100 or feebly to 1/200 dilution with such a combination of sera and reacts equally well with both, it is probably not a meningococcus. Reactions of this kind are in point of fact infrequently met with and, consequently, it is advised to regard the carriers of such cocci as suspicious and to make further examination.

(4) When the cocci under examination react with more than two of the type sera and not with normal serum.

(*a*) They are to be regarded as meningococci if they react to full titre with one of the type sera and to not more than 1/100 with any of the others.

(*b*) Cocci which give a positive reaction in presence of 1/200 dilution of three or more of the type agglutinating sera and do not react with normal serum 1/50 are probably not meningococci. The same remark, however, applies to these as to the cocci considered under 3*b*. They are but infrequently met with, and where doubt exists they should be regarded as meningococci.

TABLE I.

Reg. No. of Culture.	*Type I Serum.*			*Type II Serum.*			*Type III Serum.*			*Type IV Serum.*			*Normal Serum.*	*Glucose.*	*Maltose.*	*Mannose.*	*Saccharose.*
	1/100	*1/200*	*1/400*	*1/100*	*1/200*	*1/400*	*1/100*	*1/200*	*1/400*	*1/100*	*1/200*	*1/400*	*1/50*				
1	≠	≠	+	..	..	..	≠	(+)	..	..	..	..	..	Acid second day	Acid fourth day	..	..
9	≠	≠	+	≠	≠	≠	≠	≠	≠	≠	+	..	..	Not included in test			
10	..	..	..	≠	≠	(+)	≠	≠	(+)	≠	≠	+	..	Acid second day	Acid second day	..	..
12	≠	≠	+	≠	≠	..	≠	+	..	..	..	..	..	Acid second day	Acid second day	..	..
27	≠	+	..	≠	+	..	..	..	..	..	..	..	..	Acid second day	Acid second day	..	..
28	+	..	..	+	..	..	+	..	..	+	..	..	..	..	..	..	..
32	..	..	..	..	..	..	≠	≠	≠	≠	(+)	..	..	Not included in test			
39	≠	≠	..	..	..	..	..	..	..	..	..	..	..	Not included in test			
40	≠	≠	..	≠	≠	≠	..	..	..	+	..	..	..	Not included in test			

(5) Cocci which react to full titre, or to approximately the same titre in presence of all the agglutinating sera and give positive reactions in presence of normal serum 1/50, are not to be regarded as meningococci.

(6) Cocci which fail to agglutinate in presence of any of the sera—type agglutinating or normal—are not to be regarded as meningococci.

In the series of cocci under investigation none exhibited the characters indicated in (1); two behaved in such fashion that they could be placed in category (2). With regard to these two cocci it is to be noted that neither of them absorbed the agglutinins from any of the type sera so that we may here dismiss them to include them finally among the inagglutinable cocci—category (6).

Nine cocci of the present series fall into category (3) or (4), six into category (5), and the remainder into category (6). On saturating the type sera with those cocci which would be placed in categories (3) and (4), i. e. cocci that showed group agglutination in presence of the type sera, the results shown in Table II were obtained.

TABLE II.

TYPE I SERUM.

Reg. No. of Culture.	*Unsaturated.*				*Saturated + Homologous.*				*Saturated + Test Coccus.*			
	1/100	*1/200*	*1/300*	*1/400*	*1/100*	*1/200*	*1/300*	*1/400*	*1/100*	*1/200*	*1/300*	*1/400*
1	≠	≠	+	..	..	..	..	..	..	..	..	..
9	≠	≠	+	..	≠	≠	≠	+	..	..	..	..
10	..	..	..	..	≠	≠	≠	≠	..	..	..	..
12	≠	≠	+	..	≠	≠	≠	≠	..	..	..	..
27	≠	+	..	..	≠	≠	≠	+	..	..	..	..
28	+	..	..	..	≠	≠	≠	≠	..	..	..	..
32	..	..	..	..	≠	≠	≠	≠	..	..	..	..
39	≠	≠	..	..	..	..	..	..	..	..	..	..
40	≠	≠	..	..	≠	≠	≠	≠	..	..	..	..
Control	≠	≠	≠	≠	..	..	..	..	..	..	..	..

TYPE II 'H' SERUM.

1	..	..	..	..	≠	≠	≠	+	..	..	..	..
9	≠	≠	≠	≠	≠	≠	+	+	..	..	..	..
10	≠	≠	(+)	..	≠	≠	+	+	..	..	..	..
12	≠	≠	..	..	≠	≠	+	..	..	..	..	..
27	≠	+	..	..	≠	≠	≠	+	..	..	..	..
28	+	..	..	..	≠	≠	≠	+	..	..	..	..
32	..	..	..	..	≠	≠	≠	+	..	..	..	..
39	..	..	..	..	≠	≠	+	+	..	..	..	..
40	≠	≠	≠	≠	≠	≠	≠	+	..	..	..	..
Control	≠	≠	≠	≠	..	..	..	..	..	..	..	..

TYPE II 'F' SERUM.

1	..	..	..	..	≠	≠	≠	+	..	..	..	..
9	≠	≠	..	..	≠	≠	+	+	..	..	..	..
10	≠	+	+	..	≠	≠	+	+	..	..	..	..
12	≠	+	..	..	≠	≠	+	+	..	..	..	..
27	..	..	..	..	≠	≠	≠	+	..	..	..	..
28	+	..	..	..	≠	≠	≠	+	..	..	..	..
32	+	..	..	..	≠	≠	≠	+	..	..	..	..
39	..	..	..	..	≠	≠	≠	+	..	..	..	..
40	≠	≠	≠	+	..	..	..	..	..	..	..	..
Control	≠	≠	≠	+	..	..	..	..	..	..	..	..

TYPE III SERUM

Reg. No. of Culture.	Unsaturated. 1/100	1/200	1/300	1/400	Saturated + Homologous. 1/100	1/200	1/300	1/400	Saturated + Test Coccus. 1/100	1/200	1/300	1/400
1	≠	+)	..	..	≠	≠	≠	+	..	..		..
9	≠	≠	≠	≠	≠	≠	≠	≠	..	..	..	..
10	≠	≠	≠	(+)	≠	≠	≠	≠	..	..	..	..
12	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
27	..	..	..	..	≠	≠	≠	≠	..	..	..	..
28	+	..	..	..	≠	≠	≠	≠	..	..	..	..
32	≠	≠	≠	+	+	..	..	..	..	..	..	..
39	≠	..	..	..	≠	≠	≠	≠	..	..		..
40	..	..	..	..	≠	≠	≠	≠	..	..	..	..
Control	≠	≠	≠	≠	..	..	..	..	..	..	..	..

TYPE IV SERUM

1	..	..	..	..	≠	≠	≠	+	..	..	..	..
9	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
10	≠	≠	+	+	≠	≠	≠	+	..	..	..	..
12	+	..	..	..	≠	≠	≠	+	..	..	..	..
27	..	..	..	..	≠	≠	≠	≠	..	..	..	..
28	+	..	..	..	≠	≠	≠	≠	..	..	..	..
32	≠	≠	..	..	≠	≠	≠	+	..	..	..	..
39	..	..	..	..	≠	≠	≠	≠	..	..	..	..
40	≠	..	..	..	≠	≠	≠	≠	..	..	..	..
Control	≠	≠	≠	≠	≠	..	..	..	≠	..	..	..

From the results in this table it appears that organisms Nos. 1, 32, 39, and 40 qualify as meningococci, while the others, although they show group relationship to the meningococcus, cannot be definitely placed as members of any of the four defined epidemic strains of that organism.

The remaining 31 cocci were tested in an exactly similar manner, and it was found that none of them absorbed the agglutinins from any of the type sera.

It is seen, therefore, that, notwithstanding the fact that many of the nasopharyngeal cocci under consideration give fermentative reactions recalling those of the meningococcus, only four of the series qualify on complete investigation by serological methods as members of one or other of the four defined groups of the meningococcus.

These findings contrast strongly with the results obtained when diplococci of spinal origin are tested by similar methods, and therefore indicate that the agglutination reaction serves to differentiate meningococci of epidemic significance from other Gram-negative diplococci that inhabit the nasopharynx of man.

F. *Are the Agglutination Reactions of these Pharyngococci Constant?*

It is important that this question be considered, for it is conceivable that a coccus, although failing to agglutinate when tested after subculture for one generation, might become agglutinable after being subcultured for several generations.

The 40 micro-organisms of the series under investigation were, therefore, subcultured regularly over varying periods and were tested a second time by the standard agglutination method. All but eight behaved consistently.

The cocci whose reactions varied were Nos. 4, 5, 7, 8, 11, 13, 24, and 26. In Table III are indicated the discordant results.

TABLE III

Reg. No. of Culture.		*Type I Serum.*			*Type II Serum.*			*Type III Serum.*			*Type IV Serum.*			*Normal Serum.*
		1/100	*1/200*	*1/400*	*1/100*	*1/200*	*1/400*	*1/100*	*1/200*	*1/400*	*1/100*	*1/200*	*1/400*	*1/50.*
4	Original agglutination	..	..	..	..	..	..	..	..	..	..	..	..	..
4	Agglutination after many generations	≠	≠	..	≠	+	..	+	..	..	≠	+	..	..
5	Original agglutination	..	..	..	..	..	..	..	..	..	..	..	..	..
5	Agglutination after many generations	..	..	..	+	+	..	≠	+	..	+	..	..	..
7	Original agglutination	..	..	..	..	..	..	..	..	..	..	..	..	..
7	Agglutination after many generations	+	+	+	+	+	..	+	+	+	+	+	..	+
8	Original agglutination	..	..	..	..	..	..	..	..	..	..	..	..	..
8	Agglutination after many generations	≠	≠	..	≠	+	..	≠	+	..	..	..	..	..
11	Original agglutination	..	..	..	..	..	..	..	..	..	..	..	..	..
11	Agglutination after many generations	+	+	+	+	+	..	..	..	..	..	..	..	..
13	Original agglutination	≠	≠	+	≠	≠	..	≠	≠	+	+	..	..	≠
13	Agglutination after many generations	+	..	..	..	..	..	≠	+	..	+	..	..	≠
24	Original agglutination	..	..	..	..	..	..	..	..	..	..	..	..	..
24	Agglutination after many generations	..	..	..	+	+	+	..	..	..	..	..	..	..
26	Original agglutination	+	+	+	+	+	+	+	+	+	+	+	+	+
26	Agglutination after many generations	..	..		..			..	..			..	..	..

The behaviour of these cocci indicates that the agglutination reactions of a proportion of the Gram-negative diplococci of nasopharyngeal origin may be to some extent modified by repeated subculture.

The following points are, however, worthy of note:

(*a*) That 'inagglutinable' cocci may really bear a close relationship to those cocci which agglutinate in presence of normal serum.

(*b*) That only in two instances, No. 11 and 24, was the modification of the reaction of such a character as to lead to possible confusion. The result shown by No. 11 after prolonged sub-culture might lead one to regard that organism as a Type I meningococcus and in the case of No. 24 a Type II coccus. On neither occasion, however, did either of these cultures absorb the specific agglutinin from any of the type sera.

G. *Conclusions which may Tentatively be drawn from the Results obtained on Investigating the Series of Micro-organisms under Consideration by the standard Agglutination and Absorption of Agglutinin Technique.*

From the preceding sections of this communication the following deductions may be drawn.

1. That the majority of Gram-negative diplococci of nasopharyngeal origin which develop meningococcus-like colonies, when cultivated on trypagar, have the fermentative characters of that micro-organism.

2. Gram-negative diplococci which fail to agglutinate in presence of any of the type agglutinating sera also fail to absorb the specific agglutinins therefrom. Therein they contrast markedly with meningococci both of spinal and nasopharyngeal origin which both agglutinate in presence of, and absorb the specific agglutinin from, the type sera.

3. Those cocci which agglutinate in presence of 1/50 normal (rabbit) serum fail to absorb the agglutinins from any of the type sera and, therefore, do not qualify as meningococci.

4. Micro-organisms from the nasopharynx which react with two or more of the type sera in low dilutions seldom absorb the specific agglutinin from any of these except in those instances where the two sera with which the coccus reacts are I and III or, less frequently, II and IV.

5. Frequent subculture over long periods may be followed by alteration of the agglutination response of certain of these pharyngococci, but the modification is seldom of such a nature as to give rise to doubt as to whether a given coccus is or is not a meningococcus, provided that the suggestions offered for guidance in the interpretation of agglutination tests in Section E are borne in mind.

6. In view of the fact that all but 2 per cent. of Gram-negative diplococci of spinal origin absorb the agglutinins from one or other of the type sera, and also that micro-organisms of nasopharyngeal origin classed as meningococci because of their agglutination reactions behave in the same way, it is not improbable that the micro-organisms considered in 2 and 3 and 4 of these conclusions are not meningococci of epidemic importance at the present time.

The logical corollary to the investigation of these pharyngococci by means of the type sera prepared by inoculating animals—rabbits—with meningococci of spinal origin was to examine a similar series of pharyngococci and meningococci by the same technique, but using the serum of animals that had been immunized against representative strains of pharyngococci.

H. *Investigation of Representative Pharyngococci and Representative Type Cultures of Meningococci, using Sera of Animals—Rabbits—immunized against Pharyngococci.*

With this object in view a rabbit was immunized against culture No. 36 ; Hine's (*R.A.M.C. Journal*, October 1915) rapid method of immunization being employed. The animal was bled out on its serum attaining an agglutination titre of 1/400 with the homologous coccus.

I may note here that before immunization, the serum of this and also of the other animals subsequently used, was tested to exclude the spontaneous presence of antibodies to the micro-organisms used for immunization.

On saturating this serum with representative meningococci and pharyngococci, the results shown in Table IV were obtained.

These results show that while coccus No. 36 on being injected into an animal produces a serum which in low dilutions agglutinates the true meningococci to some extent, these organisms fail to remove from the serum its specific agglutinating property.

Another point that is worthy of note is that while culture No. 36 does remove the agglutinins from the serum, this property is not shared by any of the other pharyngococci included in the test.

In order further to investigate the question of the relationship of micro-organisms of this group to one another, culture No. 33 was used for immunizing a second rabbit. This culture failed to produce ' agglutinins ' for itself or for other cocci, although both the rapid intensive method and the prolonged method of immunization were tried.

No results, therefore, can be tabulated to indicate what relationship, if any, culture No. 33 bears to the other pharyngococci of the series under consideration.

Culture No. 38 was then used for the preparation of a third anti-pharyngococcus serum.

The agglutination response exhibited by representative cultures with this serum is shown in Table V, and the saturation results in Table VI.

This result is interesting, as it indicates a distinct relationship of some of the pharyngococci to one another, and also a degree of group relationship with certain of the meningococci, notably with specimen Type IV M.

The degree of these relationships can, to a large extent, be defined by means of the absorption test. Representative cocci—both meningococci and pharyngococci—were, therefore, used for absorbing No. 38 serum, the standard technique being employed ; the results obtained on making this investigation are shown in Table VI.

TABLE IV

ABSORPTION OF SERUM No 36

Reg. No. of Culture.	*Unabsorbed Serum*				*Absorbed + Homologous.*				*Absorbed + Test Coccus.*			
	1/100	*1/200*	*1/300*	*1/400*	*1/100*	*1/200*	*1/300*	*1/400*	*1/100*	*1/200*	*1/300*	*1/400*
Type I C.	+	..	..	..	≠	≠	≠	≠	..	..	..	..
Type I F.	≠	..	..	..	≠	≠	≠	≠	..	..	..	..
Type II F.	≠	..	..	..	≠	≠	≠	≠	..	..	..	..
Type II C.	≠	..	..	..	≠	≠	≠	≠	..	..	..	..
Type III C.	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
Type III B.	+	..	..	..	≠	≠	≠	≠	..	..	..	..
Type IV W.	(+)	..	..	..	≠	≠	≠	≠	..	..	..	..
Type IV M.	≠	(+)	..	..	≠	≠	≠	≠	..	..	..	..
No. 26	+	+	..	..	≠	≠	≠	≠	..	..	..	..
„ 27	≠	≠	≠	(+)	≠	≠	≠	≠	..	..	..	..
„ 30	+	..	..	..	≠	≠	≠	≠	..	..	..	..
„ 31	..	..	..	..	≠	≠	≠	≠	..	..	..	..
„ 33	..	..	..	..	≠	≠	≠	≠	..	..	..	..
„ 34	+	+	+	+	≠	≠	≠	≠	+	+	+	+
„ 35	+	+	+	+	≠	≠	≠	≠	+	+	+	+
„ 36	≠	≠	≠	≠	..	..	..	..	..	..	..	..

NOTE.—Cultures Nos. 34 and 35 agglutinated with all the type sera and with normal serum.

TABLE V

AGGLUTINATION OF REPRESENTATIVE MENINGOCOCCI AND PHARYNGOCOCCI BY THE SERUM OF A RABBIT IMMUNIZED AGAINST CULTURE NO. 38

	Number 38 Serum.		
Reg. No. of Culture.	*1/100*	*1/200*	*1/400*
Type I C.	+	..	..
Type I F.	+	..	..
Type II F.	≠	+	..
Type II C.	≠	+	..
Type III L.	+	..	..
Type III B.	..	..	..
Type IV W.	+	..	..
Type IV M.	≠	≠	≠
No. 17	≠	+	..
„ 25	..	..	..
„ 27	≠	+	..
„ 28	+	..	..
„ 29	..	..	..
„ 30	+	..	..
„ 31	..	..	..
„ 33	≠	≠	≠
„ 34	≠	≠	≠
„ 35	≠	≠	≠
„ 36	..	..	..
„ 37	≠	≠	≠
„ 39	..	..	..
„ 40	≠	+	..
„ 38 control	≠	≠	≠

It is seen from these results that although meningococcus Type IV M. agglutinates in presence of this serum it fails to remove the specific agglutinin from it. It may be stated that this meningococcus (IV M.) does absorb the agglutinins from a Type IV serum which also fails to agglutinate culture No. 38, similarly other meningococci failed to remove the specific agglutinin from No. 38 serum. Culture 38, therefore, cannot be regarded as exhibiting more than a group relationship to certain specimens of meningococci.

On the contrary cultures Nos. 33 and 37 are seen to be definitely related to No. 38, so that there is some evidence that the relationship between certain of the pharyngococci may be intimate. It must be noted, however, that the cultures whose reactions are shown in Table IV comprise all those of the series under consideration which did agglutinate in presence of serum No. 38 and might therefore be expected to absorb the specific agglutinin from it.

The results of the agglutination and absorption tests employing sera prepared against cultures No. 36 and 38 indicate that the pharyngococci, while exhibiting group relationship to one another, neither lend themselves readily to grouping by serological tests, nor exhibit an intimate relationship to the meningococci. It was decided, therefore, to proceed no further in the preparation of agglutinating sera against other specimens of pharyngococci, as the information already obtained seemed to justify the following general conclusions being drawn from the work done.

(*a*) If the suggestions offered for guidance in the interpretation of agglutination tests with cultures showing group relationship to the meningococcus be followed, the error of the method, when applied to the detection of carriers, will be in the direction of isolating

TABLE VI

ABSORPTION OF NO. 38 SERUM WITH REPRESENTATIVE MENINGOCOCCI AND PHARYNGOCOCCI.

NUMBER 38 SERUM.

Reg. No. of Culture.	*Unabsorbed.*				*Absorbed + Homologous.*				*Absorbed + Test Coccus.*			
	1/100	*1/200*	*1/300*	*1/400*	*1/100*	*1/200*	*1/300*	*1/400*	*1/100*	*1/200*	*1/300*	*1/400*
No. 17	≠	..	..	..	≠	≠	≠	≠	..	..	..	..
„ 27	..	..	..	..	≠	≠	≠	≠	..	..	..	..
„ 33	≠	≠	≠	≠	≠	+	..	..	..	..	..	..
„ 34	≠	≠	≠	≠	≠	≠	≠	≠	+	+	+	+
„ 35	≠	≠	≠	≠	≠	≠	≠	≠	+	+	+	+
„ 36	..	..	..	..	≠	≠	≠	≠	..	..	..	..
„ 37	≠	≠	≠	≠	≠	..	..	..	..	..	..	..
„ 40	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
Type I C.	+	..	..	..	≠	≠	≠	≠	..	..	..	..
Type II C.	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
Type II F	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
Type III C.	≠	+	..	..	≠	≠	≠	≠	..	..	..	..
Type IV M.	≠	≠	≠	≠	≠	≠	≠	≠	..	..	..	..
No. 38	≠	≠	≠	≠	..	..	..	..	..	..	..	..

a small number of men who are not meningococcus carriers, and not in the discharge of men who are.

(*b*) The examination of pharyngococci shows that many of them may sufficiently resemble the meningococcus to be mistaken for that micro-organism if morphological and cultural characters—including fermentation reactions—be alone relied upon for the purpose of identification.

(*c*) A point of importance is that the pharyngococci do not readily lend themselves to grouping by serological methods, as do meningococci of known pathological significance. Therein they resemble other non-pathogenic bacteria.

(*d*) While group relationship between the pharyngococci and meningococci cannot be denied, that relationship appears to be remote, and, if care be taken, should not lead to confusion in the interpretation of results obtained by the agglutination test.

It is important that the significance of these facts be appreciated, as the only alternative method that can be used at present for differentiating meningococci from other Gram-negative diplococci is the application of the fermentation tests.

The assessment of results when bacteria are investigated by the carbohydrate tests calls for exceptional skill in bacteriological technique, and occupies so much time that at present the more rapid method of serological examination is almost a military necessity.

Moreover, one of the most valuable sugars for differential purposes in the investigation of Gram-negative cocci is maltose, and I am informed by Professor Irvine that there is grave doubt as to whether maltose is or is not a chemical entity.

I. *Agglutination of Representatives of the present Series of Cultures with Polyvalent Antimeningococcus Horse serum, prepared for Therapeutic Purposes.*

As a considerable number of observations have been made in which polyvalent therapeutic antimeningococcus serum has been used for differentiating meningococci from other Gram-negative cocci found in the nasopharynx, it appeared of some importance to compare the results so obtained with those in which specific univalent type (rabbit) serum was employed, using the standard technique.

Results obtained with representatives of the series of cocci under examination are shown in Table VII.

It would be anticipated that cocci which showed group agglutination with the univalent (rabbit) sera, or reacted in presence of normal rabbit serum, would give a positive result with the more potent horse serum ; while if the result given by the horse serum be really specific, negative results would be expected with those cocci which fail to react in presence of any of the type agglutinating rabbit sera.

It will be seen from the above table that of eight cocci which gave negative results when tested with the rabbit sera, five react markedly with the horse serum, one reacts feebly, and only two are negative.

It would, therefore, be quite *safe* to make use of such therapeutic serum for the detection of meningococcus carriers, but such procedure would result in a comparatively large number of men being segregated

who were not carriers of any of the defined types of that micro-organism. The use of therapeutic serum for diagnostic purposes is therefore not advisable.

TABLE VII.

Registered Number of Culture.	*Description of Reaction of Coccus when tested by the standard method.*	*Result of Agglutination with Therapeutic Serum.* 1 : 100	1 : 200	1 : 400
17	This organism failed to agglutinate with any rabbit serum	≠	..	..
25	,, ,, ,, ,,	≠	≠	≠
26	Agglutinated both in presence of type sera and normal serum	≠	+	(+)
27	Agglutinated to 1:200 with Type I and Type II (Table I)	≠	≠	≠
28	Agglutinated to 1 : 100 with all four type sera	..	..	..
29	Failed to agglutinate in presence of any of the type sera	≠	≠	≠
30	Agglutinated both with type sera and normal serum	≠	≠	≠
31	Failed to agglutinate in presence of any of the type sera	..	..	..
32	Proved to be a Type III coccus by agglutination and absorption	≠	≠	≠
33	Failed to agglutinate in presence of any of the type sera	≠	≠	+
34	Agglutinated both with type sera and normal serum	≠	≠	≠
35	,, ,, ,, ,,	≠	≠	≠
36	Failed to agglutinate in presence of any of the type sera	..	..	..
37	,, ,, ,, ,,	≠	≠	≠
38	,, ,, ,, ,,	≠	≠	≠
40	Proved to be a Type II by agglutination and absorption	≠	≠	≠

J. *Conclusions.*

1. A considerable number of Gram-negative cocci of nasopharyngeal origin, which resemble the meningococcus morphologically, culturally, and in fermentative reactions, fail to qualify as members of any group of that micro-organism on serological examination.

2. Provided that the source of error in technique and in interpretation of results are properly appreciated, the agglutination test can be satisfactorily employed as a practical measure for the detection of meningococcus carriers.

3. Certain of the Gram-negative diplococci of nasopharyngeal origin which fail to qualify as meningococci show, on serological examination, group relationship to one or other of the defined types of that micro-organism. They also show relationship to one another, but do not lend themselves so readily to grouping by serological methods as do meningococci of known pathogenic significance.

4. It is inadvisable to employ polyvalent therapeutic serum derived from the horse for the purpose of differentiating meningococci from other Gram-negative diplococci.

PART III. APPLICATION OF THE PRECEDING AND FURTHER RESEARCHES TO THE PREVENTION, DIAGNOSIS, AND SPECIFIC TREATMENT OF CEREBROSPINAL FEVER

19. The Prevalence and Distribution of Individual Types of the Meningococcus.

A. Meningococci isolated from the Cerebrospinal Fluid of Cases of Cerebrospinal Fever.

Between October 1915 and April 1919 a register was kept of the serological characters of all meningococci received at the Central C.S.F. Laboratory. An analysis of the results obtained during this period of three and a half years gives the following results.

(*a*) *Proportion of Meningococci identified by the Agglutination Test.*

Total number of meningococci from C.S. fluid examined, 526.
Number identified as specimens of one or other of the 4 types, 518.
Number unplaced or doubtful, 8.

No less than 98 per cent. of all the meningococci received, therefore, have been relegated by the agglutination test to one or other of the four types.

(*b*) *The Frequency of each Type.*

The results are as follows :

Type	I.	II.	III.	IV.
Specimens	195	218	69	36
Percentage	37·66	44·05	11·38	6·94

80 per cent. of the meningococci, therefore, were included in the first two groups. Although Type II was on the whole the most common, Type I approaches it closely in frequency. The above figures refer to cases occurring between October 1915 and April 1919. The figures for 1915 alone were as follows :

Type	I.	II.	III.	IV.
Specimens	31	20	10	2
Percentage	49	31	15	3

from which it is seen that during 1915 also 80 per cent. of the meningococci were included in the first two groups, but during that year Type I meningococcus was more abundant than Type II among the 98 specimens of meningococcus submitted to serological examination.

An Example of the Local Prevalence of an uncommon Type of the Meningococcus. At a comparatively early stage of the observations

it was noticed that the relative frequency of individual types of the meningococcus is not the same in every district, and that certain localities may show a relatively high frequency of a particular type. The frequency of Type III observed by Dr. P. C. Laws at Newcastle is a case in point. The types of meningococcus isolated from cases among the military and civilians respectively at Newcastle during the four years 1916–19 are seen in the following figures kindly supplied by Dr. Laws:

	Military.				*Civilians.*			
Type	I.	II.	III.	IV.	I.	II.	III.	IV.
1916	26	8	3	0	2	2	1	1
1917	16	32	4	0	0	2	1	0 *
1918	18	9	9	0	0	4	5	0
1919	0	2	0	0	3	1	2	0
Total	60	51	16	0	5	9	9	1

* Figures incomplete.

The percentage of individual types amongst military and civilians respectively during the period in question therefore was :

Source.	*Specimens.*	*Types*			
		I.	II.	III.	IV.
Military	127	47·2	40·1	12·5	0
Civilians	24	20	37·5	37·5	4

The frequency of Type III among the civilians is especially striking, and amongst the military it would appear to be above the average of 11·38 found among specimens of meningococcus received from all parts of the country during this period. On examining the yearly type rates at Newcastle it is seen that Type III was especially prevalent during 1918, in which year over half the civilian cases and a quarter of the military ones yielded a meningococcus of this type.

(*c*) *Sources of these Meningococci.*

Of the 518 meningococci identified at the Central C.S.F. Laboratory 491 were isolated from cases in the United Kingdom, 462 coming from adults chiefly soldiers, and 29 from children. In addition, 18 specimens of meningococcus were received from South Africa, and 9 from the U.S.A. The former were kindly sent by Captain Pullon, the latter by Dr. McCoy. Captain Pullon had independently investigated his specimens of meningococci, and had found them to conform to one or other of three different serological types, a result confirmed by our tests. The cocci from abroad were received in the form of saline suspensions which had been duly heated and phenolated. Particulars with regard to these meningococci are seen in the following table :

	United Kingdom.		*South Africa.*	*U.S.A.*
Type.	*Adults.*	*Children.*		
I	175	8	9	3
II	194	15	6	3
III	62	2	3	2
IV	31	4	0	1
Total	462	29	18	9

It will be observed in the first place that the meningococci from children, many of whom were clinically cases of post-basic meningitis, are included within the four types equally with those coming from adults, and that among the South African specimens were examples of three types, while among the U.S.A. meningococci all four types were represented. Whatever the source, the majority of the meningococci are included in the first two groups. Type II would appear to be commoner than the other types in children, so far as can be seen from the comparatively small number of meningococci from children examined. In case of the South African specimens Type I was in chief abundance, while among the U.S.A. specimens Types I and II were equally represented.

Since the specimens of meningococcus received from abroad failed to provide examples of any further types, it would appear that these four serological types of the meningococcus have a wide geographical distribution.

(d) Result of the Absorption Test.

No less than 235 of the meningococci that were placed definitely in one or other of the four serological groups by the agglutination test were subsequently submitted to the absorption test. The results were as follows :

	Type by Agglutination.	*Type by Absorption.*	
Type.	*Specimens.*	*Confirmed.*	*Not confirmed.*
I	72	70	2
II	87	87	0
III	43	42	1
IV	33	32	1
Total . . .	235	231	4

These results are striking evidence of the accuracy of the agglutination test when carried out in a strictly quantitative fashion, and with suitable controls, by the standard procedure practised throughout.

(e) Result of the Examination of a Plurality of Colonies in Primary Cultures from the Cerebrospinal Fluid of Cases of C.S. Fever.

In order to see if more than one type of meningococcus occurred in a primary culture from the cerebrospinal fluid of a case, from two to four colonies were subcultured from the primary plates made from the cerebrospinal fluid of 26 cases of C.S. fever. The colonies were purposely chosen from parts of the plate as widely separate as possible. In each individual case the serological result given by the cultures from the different colonies were identical, and they provide therefore substantial evidence in favour of the view that in at any rate the vast majority of instances only a single type of meningococcus occurs in a case of cerebrospinal fever.

B. Meningococci isolated from Nasopharyngeal Secretion.

(a) The Nasopharyngeal Secretion of Cases.

The view of Netter and Debrè, based on the evidence available in 1911 as to the constancy of the meningococcus in the nasopharyngeal secretion at the onset of cerebrospinal fever, has already been men-

tioned. It was of interest, therefore, to obtain further evidence on this matter, and especially to determine if the meningococcus lodged in the nasopharynx of the patient is of the same serological type as that present in his cerebrospinal fluid. These points were carefully examined by Flack, who found that the meningococcus is constantly present in the nasopharynx of the patient at the onset of cerebrospinal fever, provided that a very thorough search is made for it; and secondly, that in 31 out of 32 patients examined by him the meningococcus in the nasopharynx was of the same serological type as that present in the patient's cerebrospinal fluid. This same point was investigated independently by Andrewes and Canti, who found that in all of 25 cases of cerebrospinal fever examined by them in the acute stage—10 of which cases were in children under 2 years of age—the meningococcus was present in the nasopharynx, and was of the same serological type as that present in the cerebrospinal fluid. W. M. Scott also found the meningococci to be similar in the two situations in six out of seven cases examined by him. Out of ten cases in which meningococci from the nasopharynx and cerebrospinal fluid of the same patient were forwarded to the Central C.S.F. Laboratory they were found in each patient to be identical, although these cases purposely included patients infected by meningococci of each of the four serological types. So close was the correspondence that even slight peculiarities in the agglutinability of the cerebrospinal fluid strain were found to be faithfully reproduced by the strain isolated from the nasopharynx. Embleton also observed a similar identity of type in 35 out of 36 cases of cerebrospinal fever examined by him. It may be taken, therefore, that at the onset of cerebrospinal fever the meningococcus is constantly present in the nasopharyngeal secretion of the patient, and that as a rule it is of the same serological type as that present in the cerebrospinal fluid of the case.

With regard to this question of the presence of the meningococcus in the nasopharyngeal secretion of the patient, it is of interest to recall that Fildes had the unique experience of examining the nasopharyngeal secretion before the onset of symptoms in no less than 26 of his cases. These tests were performed on each individual at an interval varying between 2 and 75 days before the onset, and the result in every case was negative. This observation is of considerable importance, because as Fildes points out it indicates that cases are seldom carriers before the onset, and that the period of time intervening between the acquisition of the coccus and the onset of the disease must usually be short. At the same time, the constant presence of the meningococcus in the nasopharyngeal secretion at the onset of cerebrospinal fever brings cases and carriers into line, so that every case of cerebrospinal fever may be regarded as an instance of a carrier developing the disease at the onset of his period of carrying.

Fildes was less fortunate than the authors mentioned above in recovering the meningococcus from the nasopharyngeal secretion of patients suffering from cerebrospinal fever, since out of 34 cases examined by him 13 were negative in spite of repeated examinations. The first swab, however, was taken by him on the average nine days

after the onset ; in the negative cases eleven days, and in the positive eight days. Fildes's results, therefore, do not exclude the presence of the meningococcus in the nasopharynx at the onset ; they would appear rather to suggest the tendency of the coccus to disappear or intermit in a minority of his cases after a comparatively short time. Embleton has recently emphasized this tendency of the meningococcus to intermit in the nasopharynx of cases. As regards the nature of the type in the nasopharynx and cerebrospinal fluid respectively of the same patient, Fildes found that in 15 of his cases the types in both situations were the same, but in six cases the nasopharynx yielded a different type to that present in the cerebrospinal fluid.

Embleton, working at Netley, has made a very careful study of the duration of carrying by patients convalescent from cerebrospinal fever, and has found that it is of the utmost importance to apply the swab with appreciable pressure to the nasopharyngeal mucosa in order to obtain a positive culture, since the meningococcus appears frequently to lurk in the deeper layers of the mucous membrane. He emphasizes also the tendency of convalescent carriers to be intermittent. Out of 97 C.S.F. convalescents at various stages received at Netley from overseas 66 proved to be carriers. It is of great significance that all of 38 cases of C.S. fever that Embleton had the opportunity of examining throughout their period of convalescence were found by him to be chronic carriers, the average period of carrying being 6 months. He found also that a convalescent case carries only one type of meningococcus throughout, and he met with but one exception to this rule in all his very numerous examinations of them.

(*b*) *The Nasopharyngeal Secretion of those in the Environment of the Case.*

The following instances in which the distribution of types of the meningococcus amongst cases and those in their environment could be compared are instructive.

Owing to the fact that drafts from a certain large garrison had carried cerebrospinal fever over to France, a very extensive swabbing of troops in this garrison was undertaken with a view to preventing further transference of the disease. Meningococci from thirteen cases of cerebrospinal fever in this garrison at the time showed the following distribution of types of the meningococcus :

Type . .	I	II	III	IV
Specimens .	3	5	0	5

Captain R. R. Armstrong swabbed no less than 10,000 men in this garrison and isolated 410 of them as carriers of an organism closely resembling the meningococcus. Of these 86 had become free of suspicious organisms when they were examined some days later by Major W. J. Tulloch. All of the remaining 324 yielded meningococcus-like organisms which were submitted to serological examination with the three monotypical sera found to include the meningococci present in the cerebrospinal fluid of the cases. The number of men found by

Major Tulloch to be carrying meningococci in their nasopharynx was 173, and the distribution of types among them was as follows :

Type	I	II	IV
Specimens	30	72	71

The grouping of types in cases and in carriers in this garrison, therefore, corresponded to a remarkable degree. The large proportion of both cases and carriers infected by Type IV was a very exceptional feature of this outbreak.

Colonel Flack observed a similar correspondence between the serological types of meningococcus carried by cases and contacts with them in the London District during 1916. The figures obtained by him were as follows :

Source	Type I	Type II	Type III	Type IV	Total.
Cases	2	13	0	1	16
Contacts	13	78	0	1	92

It is noteworthy that here Type II was six times as frequent as Type I both in cases and in carriers.

Further observations on the distribution of types of meningococcus among cases, and those in their environment, will be found in the section by Captain Glover on the carrier rate, where some striking evidence of the correspondence in type distribution in cases and in those in their neighbourhood will be seen. It would seem from these observations that as a rule the type of meningococcus present in cases among troops is the same as that prevalent in their vicinity.

Fildes, when investigating the type of meningococcus carried by those in the environment of 23 cases of cerebrospinal fever among naval ratings in barracks distinguished between close contacts and remote contacts. Out of 31 carriers found among 297 close contacts 15 carried a coccus of the same type as the case, while 16 carried a different type. Out of 809 remote contacts 17 carried a type similar to the case, but 45 carried a different type. As Fildes points out these figures show that a considerable proportion of the carriers in the community examined by him had nothing to do with the case. The fact that the carrier rate of the same type as the case was relatively double as high (5 per cent.) in close contacts as in remote contacts (2·1 per cent.), while the carrier rate of a different type to the case was practically the same in both close contacts (5·4 per cent.) and in remote contacts (5·5 per cent.) is suggestive.

20. Observations of the Meningococcus Carrier Rate, and their Application to the Prevention of Cerebrospinal Fever.

By J. A. Glover, O.B.E., M.D., D.P.H.,

Late Captain R.A.M.C., and Officer in Charge of the London District Cerebrospinal Fever Laboratory.

A. *Definition of Terms used in this Paper.*

Overcrowding occurs when there is the slightest excess in numbers over ' Mobilization ' standard, i. e. when each man has an average of less than 40 sq. ft. of floor space.

Severe overcrowding occurs when the edges of beds are less than one foot apart. (*Note*, the Army bed is 2½ ft. wide.)

Meningococcus = a Gram-negative diplococcus culturally and morphologically resembling meningococcus and showing definite macroscopic agglutination up to at least half the full titre (when kept in contact for 24 hours at 55° C.) with one or other of the four monotypical agglutinating sera supplied from the Central C.S.F. Laboratory.

Contact = a person who has slept in the same tent, hut, or barrack-room, with a patient before the onset of the disease, or who has been within 3 yards of him under conditions likely to favour infection.

B. *Introduction.*

Much work has been done in the London C.S.F. Laboratory upon the variations in the carrier rate amongst soldier non-contacts, as well as amongst contacts.

An important part of its work has consisted in regular observations of the carrier rate of the same military community, the Guards Dépôt at Caterham, by means of almost weekly examinations of samples of 100 men.

More than 12,000 examinations of non-contacts were made at Caterham.

Moreover, during the latter part of 1917, and throughout 1918, this bacteriological watch upon the carrier rate was extended to many other units of the London Command, some of them, especially some of the reserve battalions of the Foot Guards, being kept under a more or less regular observation.

In addition to their bearing upon the aetiology of cerebrospinal fever, these observations gain interest from the fact that by tracing the spread of so distinctive an organism as the meningococcus verified in every case by agglutination and divided into several types, it is possible to study certain phenomena, probably widely true of many other kinds of respiratory infection, and revealed in these questions of carrier rate.

It has seemed right, therefore, that these phenomena should be again briefly discussed.

C. *The Close Relation between Overcrowding and Carrier Rate.*

At an early stage of the recent outbreak, Walker Hall, Johnstone, and Treadgold, and several other observers had pointed out the connexion between the degree of overcrowding and the number of positive contacts detected in the investigation of contacts of actual cases of the disease, but the relationship was now found to extend equally to those in the same community who had not been in any known contact with actual cases of the disease.

Some of the earlier results have already been reported to the Medical Research Committee in a Special Report printed March 9, 1918, and the later observations, with scarcely an exception, confirm the former ones.

In briefly summarizing the results of the observations, some of the points in the former report will of necessity be repeated.

D. *Military Overcrowding in the War Emergency.*

It should be remembered that the peace or standard accommodation of the British Army was laid down by Royal Commission in 1861, after the Crimean War, and was fixed at 60 sq. ft. of floor space, 600 cubic ft. air space, and 3 ft. distance between beds.

In the extreme pressure following the outbreak of this war, permission was granted to use a mobilization standard of 40 sq. ft. floor space and 400 cubic ft. air space, but nothing was said as to the distance between the beds.

Mobilization standard means as a rule an increase of 50 per cent. in the number of men allowed in barrack-room or hut ; 36 instead of 24 in the former, 30 instead of 20 in the latter.

Even this mobilization standard was almost immediately regarded as an unattainable ideal (although it was the lowest possible limit of hygienic safety), and was from the first more honoured in the breach than in the observance.

Again, it looks like a simple deduction that if the floor space be reduced from 60 sq. ft. to 40, the distance between the beds will be reduced from 3 ft. to 2 ft., but it actually happens that in the ordinary barrack-rooms or huts, where sleeping is not feasible, except along the sides, the reduction is more than 50 per cent.

The distance between beds in an ordinary barrack-room on mobilization standard averages 1 ft. 4 in., after allowing for fireplaces, cupboards, and doors ; and any beds over this standard reduce it very rapidly.

Thus, in an actual instance after three years of war, where the peace-strength of a barrack-room was 24, 47 men were found to be sleeping in it, and the beds instead of being $\frac{3 \times 24}{47}$, or $1\frac{1}{2}$ ft. apart, were actually less than 6 in. apart ; this, too, in a most distinguished and efficient unit.

Most of the huts erected during the war were of two main types, the first 60 ft. by 20 ft., the second 60 ft. by 15 ft. With the first type (60 ft. by 20 ft.), ' mobilization ' standard means thirty men, and (after allowing for the stove in winter) about 1 ft. 4 in. between beds ; whereas, with the second type (60 ft. by 15 ft.), ' mobilization ' standard (often transgressed with this type), meant twenty-two men, and at least 2 ft. 6 in. between beds.

Assuming equal supervision of ventilation and rigid adherence to mobilization standard in this respect, the smaller pattern scored heavily. But it was this fatal beauty of space between the beds that afforded great temptation to hard-pressed and hygienically unprincipled authorities to put in more beds, and so to overcrowd ; and the 60 ft. by 15 ft. huts were therefore more often in excess of mobilization than their larger compeers.

Whilst, therefore, we may for convenience define severe overcrowding as occurring in sleeping quarters when the edges of beds are less than one foot apart, it is obvious that overcrowding is more severe when the beds of a 60 ft. by 15 ft. hut are within 1 ft. of each other than when those of a 60 ft. by 20 ft. hut are at the same distance, whilst the conditions in the latter will in turn be worse probably than those in a barrack-room, though here again much depends upon the

design and date of the latter. Also, it must be remembered that in nearly all the sleeping accommodation examined (i. e. before the Armistice) ventilation had been seriously interfered with by compliance with anti-aircraft lighting restrictions, by the use of blankets as extemporized curtains, or even by keeping the windows (painted black) shut, as was done in the barrack-room occupied by 47 men just referred to.

E. *Overcrowding in Sleeping Quarters a factor of Paramount Importance in the Production of High Carrier Rates.*

Overcrowding in sleeping quarters is of paramount importance for several obvious reasons.

First, the relative position of the infecting carrier and the infected man and their close proximity are unchanged for many consecutive hours.

Secondly, the movement of air in the room is much less than during the daytime, both from absence of movement of the occupants and by the closure of doors and windows.

Thirdly, the great majority of persons sleep open-mouthed, so that the nasal filter is short-circuited, and the recipient receives the droplets of secretion containing the infection, and sprayed by the cough or sneeze of the carrier, straight on to the posterior portion of the naso-pharynx.

Neither the salivary flora (Gordon), nor the flora of the anterior nares is favourable to the growth of the meningococcus.

That infection does take place during sleep is strongly suggested by bed-charts of the rooms or huts occupied by the men examined.

Chart I (kindly reproduced by permission of the Editor from the *Journal of Hygiene*, vol. xvii, Oct. 1918) shows a number of carriers sleeping in little congeries of the same type in closely adjacent beds, and the same phenomenon is seen in Chart I of my special report to the Medical Research Committee of March 9, 1919, in the case of half a hut.

Such grouping of carriers is more suggestive of conditions conducive to the spread of infection than the same number of carriers more uniformly distributed.

These and many other bed-charts suggest that, whilst, of course, infection undoubtedly takes place in crowded canteens, cinemas, and even in crowded medical inspection rooms sometimes, proximity during sleep is of the first importance. For this reason, too, transports and long-distance troop trains have been great agents in the spread of infection.

Moreover, as negative evidence of the importance of sleeping contact may be adduced the fact that several samples of men working in very overcrowded offices or workshops, but sleeping in fair billets, showed comparatively low carrier rates.

Thus, for example, 52 soldier clerks were examined who were working in what was probably the worst office accommodation in London, a warehouse of many floors, no through ventilation, and what ventilation there was extremely imperfect, the atmosphere nauseating to the freshly entrant nose. The average floor area per

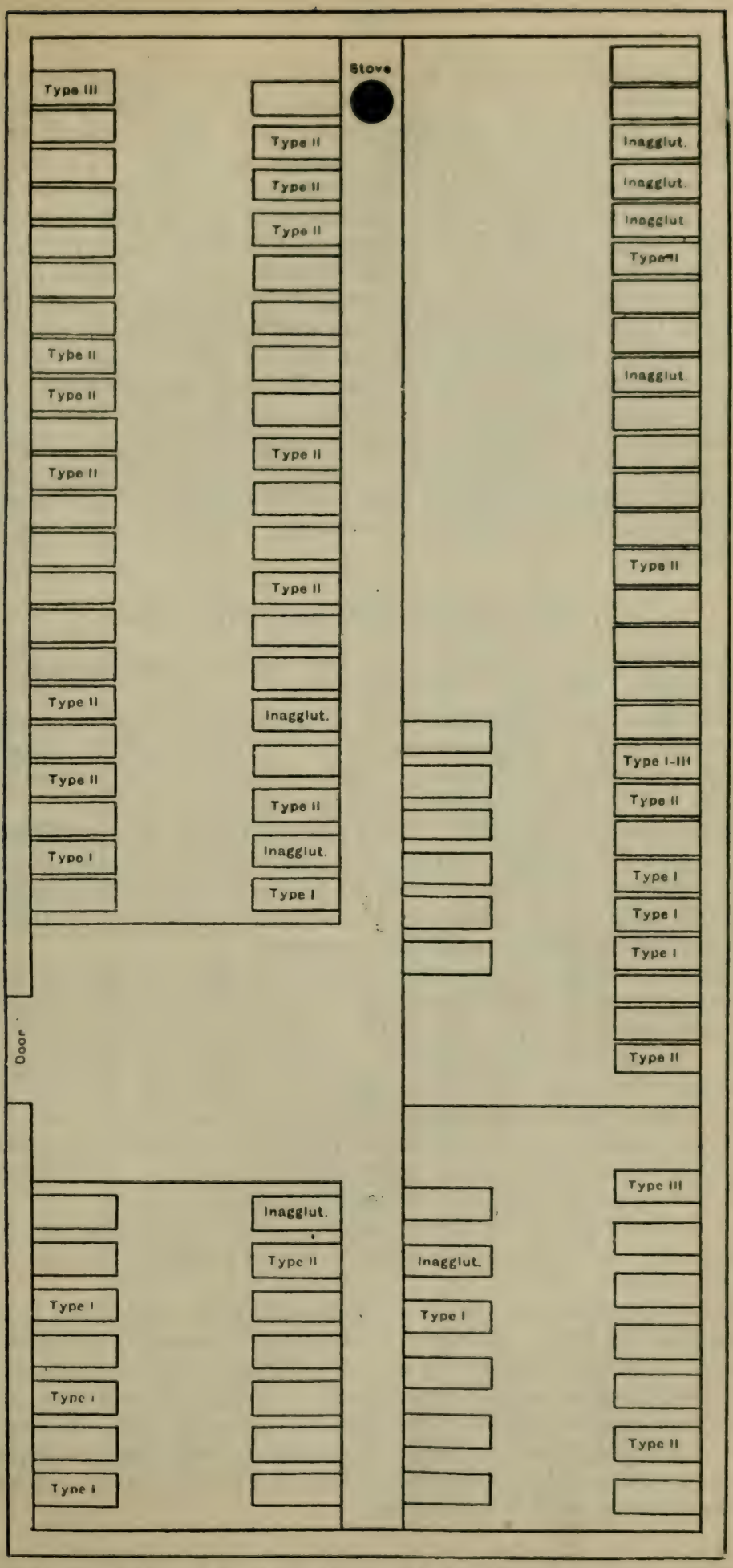


CHART I.—Cross infection due to proximity of beds. (Drawn to scale; each bed is $2\frac{1}{2}$ ft in width.)

clerk appeared to be 22 sq. ft. instead of the 50 sq. ft. laid down by the Barrack Schedule. In other words, the Barrack Schedule of 50 sq. ft. per clerk would have allowed 416 clerks to work in the building, and there were actually working in it 925. It is obvious there was much overcrowding, bad ventilation, and there had been much previous influenza. The season was the end of November 1918. A high carrier rate would have appeared natural, if not inevitable, but the 52 soldiers only yielded three carriers. The reasons for this low rate (6 per cent.) were apparently that all slept in good billets, many in their own homes, that there was a great dilution with lady civilian clerks, and that the tables at which work was done were very broad, ensuring 3 ft. at least between clerks facing each other.

The danger of sporadic cases arising in such an office is, of course, considerable. One civil community investigated after the occurrence of a case showed a high carrier rate despite excellent sleeping accommodation. Here the infection undoubtedly took place in a day-room crowded for several hours each evening. Here, too, influenza had been rife.

F. *Space between Beds and Carrier Rate.*

The actual space between beds and efficient ventilation are the vital points, ' wall space ' being essential.

Mere floor space and mere cubic space are not at all of the same importance. The cubic space at one well-known dépôt, for example, was almost infinite, and yet here infection was rife, owing to proximity of beds, which were arranged in blocks, 4 in 64 sq. ft.

In the London Command, taking barrack-rooms and huts together, it was found that strict adherence to a mobilization standard usually gave a carrier rate of just under 10 per cent.—that is, those agglutinating with one or other of the standard type serums of Gordon. Epidemic meningococci only were counted.

This (with ordinary barrack ventilation modified by lighting restrictions always understood) meant that a distance of about 1 ft. 4 in. between beds corresponded with a carrier rate of about 10 per cent. A very slight excess above ' mobilization ' very rapidly sent up the carrier rate to between 10 and 20 per cent. If the beds were closer than 1 ft., 20 per cent. was almost invariably found, and if the distance were under 9 in., 28 to 30 per cent. was usual. A ' peace ' standard of accommodation, unless there were some gross defect of ventilation, rarely yielded a carrier rate over 5 per cent.

So definite was this relation under war conditions that one could almost determine the carrier rate of a barrack-room with a foot-rule, see Chart on p. 139.

For evidence we may take twelve months from September 1, 1917, to August 31, 1918. This period is selected because it includes two periods of pressure upon the accommodation of the Command, and because a greater number of non-contacts were examined than during any other twelve months. The number of actual cases during this period was very small, probably in part due to strenuous prophylactic efforts to reduce high carrier rates, when such were found, and partly to the subsidence of the epidemic.

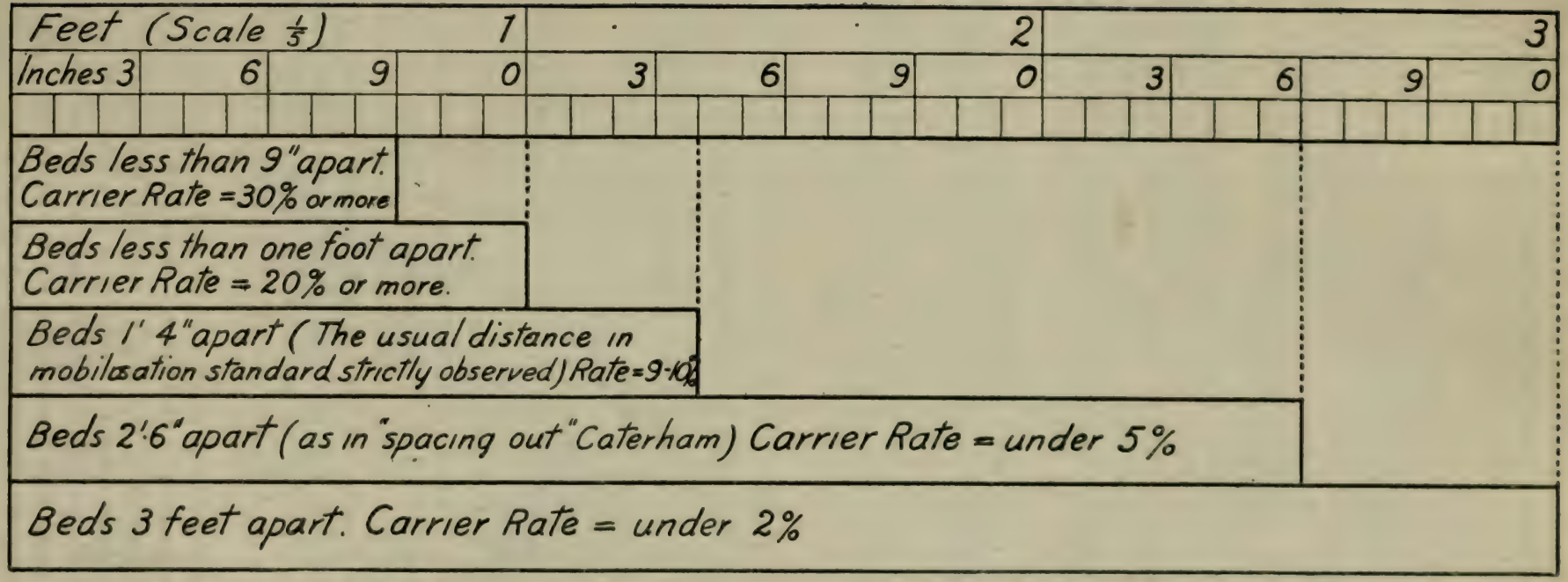


CHART II.—Relation of distance between edges of beds to carrier rate. Army plank beds 2½ ft. wide in ordinary barrack-rooms and huts under war conditions. (*Note*.—The overcrowding must have existed for three weeks.)

During this twelve-month period, 93 samples (each of an average of 100) non-contact men were examined with the following results :

Twelve samples showed carrier rates of 20 per cent. or over : in every unit of these save one there was existent at the time of the examination, or shortly before, *gross* overcrowding.

The single exception was in one block of a London barracks, built about 1816, in which the ventilation and general hygienic conditions were so defective that the most thorough spacing-out failed to reduce the carrier rate for a long time. Four cases occurred during the twelve months in this single block alone, a sixth of all the cases in the Command during this twelve months.

Such carrier rates were considered signals for prompt and effective action to abate overcrowding and to increase ventilation.

Seventeen samples showed carrier rates of 11 to 19 per cent. In 14 of these there had been, or was, some overcrowding or other obviously unsatisfactory hygienic condition. Many of these were from units housed in adapted premises, pavilions, breweries, and even horse-boxes.

These carrier rates were considered unsatisfactory.

Thirty samples showed carrier rates of from 6 to 10 per cent.

Thirty-four samples showed carrier rates of 5 per cent. or under.

Of these two latter classes all (save two) showed good hygienic conditions, the 34 with carrier rates of 5 or under being all units who had been ' spaced out ' for some time.

G. ' *The Warning Rise* ' *in the Non-contact Carrier Rate.*

It was noted also that if overcrowding occurred in a unit which had not previously been overcrowded, two things happened : First, a marked rise in the carrier rate occurred, usually within a fortnight, reaching its maximum about three weeks after the overcrowding began ; this is termed the ' warning rise '.

Secondly, the proportion of carriers of epidemic or agglutinable strains of the meningococcus to carriers of inagglutinable strains very markedly increased.

A sharp rise in the non-contact carrier rate to over 20 per cent. is as sure a storm signal of imminent danger of an outbreak as it is a sign of overcrowding or of dangerously deficient ventilation.

The development of this carrier rate wave is accelerated by coexistent catarrhal diseases which act as intensifiers to the overcrowding.

A similar warning rise probably takes place in some other diseases such, for example, as diphtheria (cf. ' A Study of Diphtheria Carriers in a Military Camp,' Keefer, *Journal of American Medical Association*, Oct. 12, 1918).

Whilst overcrowding produced a rise in the carrier rate very quickly, a return to mobilization standard was not sufficient to reduce the carrier rate markedly, but the carrier rate was reduced with remarkable success by spacing out the beds to 2½ ft. distance between each, although not at so fast a rate as overcrowding will raise it. To raise the carrier rate by overcrowding was both easier and quicker than to diminish it by spacing out.

Thus, Caterham dépôt, which had been purged by six months of

spacing out, and which had a carrier rate for the month of March (1918) of 0·5 per cent., was severely overcrowded in April (beginning on April 20). By May 2 the carrier rate was 34 per cent.

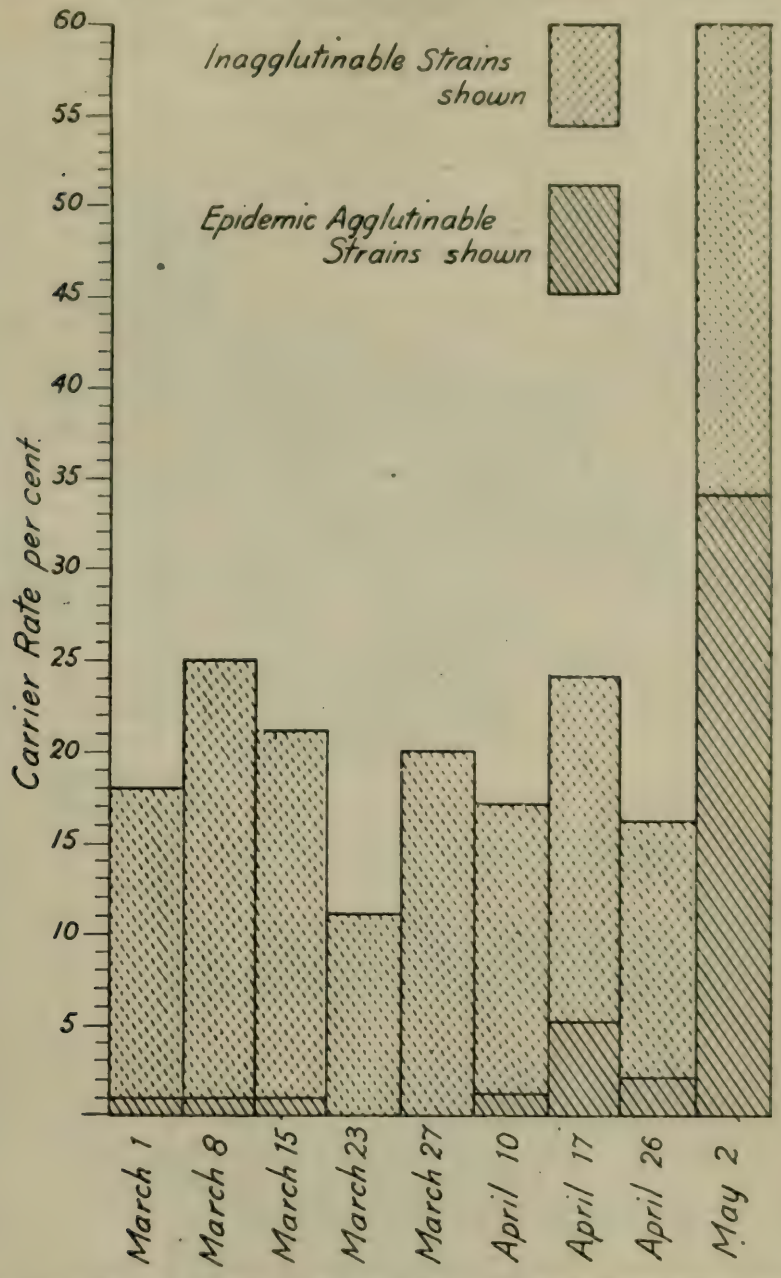


CHART III.—Showing the second danger signal: the rise in proportion of epidemic agglutinable strains to inagglutinable strains subsequent to severe overcrowding and just preceding an outbreak. (*Note.*—Moderate overcrowding commenced, April 20, 1918. Severe overcrowding commenced, April 25, 1918. First case occurred, May 7, 1918. Spacing-out in force from October 4, 1917 to April 19, 1918.)

The proportion of carriers of epidemic strains to carriers of inagglutinables which had been $\frac{5}{19}$ on April 17 and $\frac{3}{13}$ on April 26 rose to $\frac{34}{26}$ on May 2, i.e. 13 days after overcrowding had begun. The first actual case of the disease was diagnosed on May 9.

This is illustrated on Chart III (Caterham, 1918) and on Chart IV (Caterham, 1917) (the latter reproduced from the *Journal of Hygiene* by kind permission).

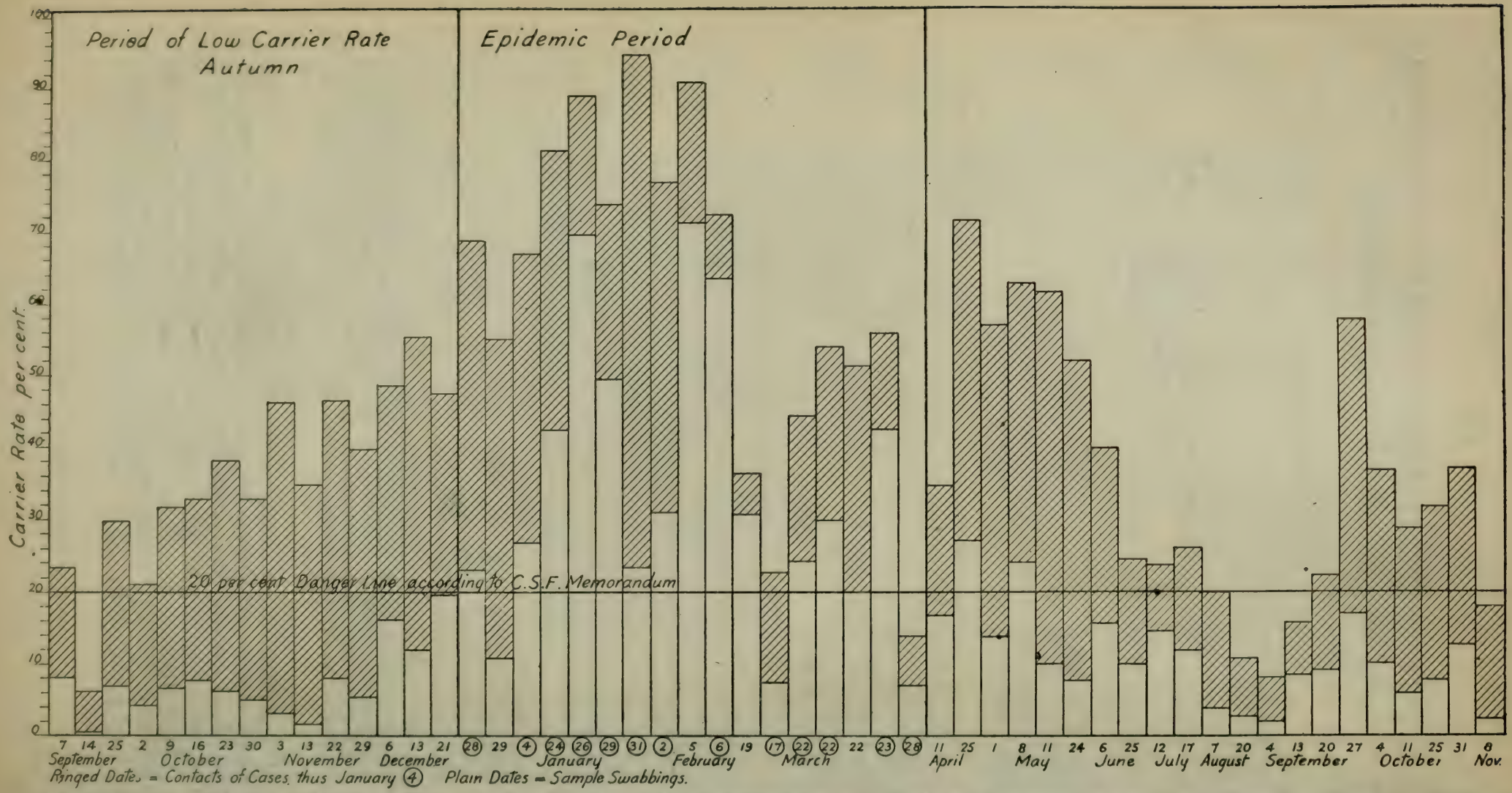


CHART IV.—1917 epidemic, cerebrospinal fever. Caterham Dépôt. Relative percentages of: (1) Carriers of Gram-negative cocci morphologically indistinguishable from meningococci but not qualifying serologically as such—shaded columns (2) Carriers of meningococci serologically proved—plain columns. Showing the greatly increased proportion of the latter during the epidemic period.

This rise in the proportion of epidemic strains constitutes a second danger signal.

Caterham Dépôt, whence these two illustrations come, and where the non-contact carrier rate (estimated by weekly samples of approximately 100 men) was under observation for nearly three years, showed admirably the phenomenon of the warning rise on two occasions, and afforded a remarkable demonstration of the relation of overcrowding to carrier rate, in that it suffered two periods of overcrowding alternated with a period of spacing out.

I propose, therefore, to recapitulate the cerebrospinal fever history of Caterham Dépôt from the beginning of the War down to the signing of the Peace Treaty.

Caterham Dépôt. In peace-time the Dépôt is housed entirely in its permanent barrack-rooms and has a normal population of some 800 men. Since war began, its accommodation has been increased to fivefold by huts, and the population has sometimes exceeded 13,000 by the use of tentage.

The Dépôt receives straight from civil life the recruits of the five regiments of Foot Guards. These recruits it trains for three months intensively, and they are then drafted to the respective reserve battalions of the five regiments in various places. Most of the population is, therefore, floating: it abides three months, and passes. There is, however, a permanent staff of instructors, and two trained soldiers are in each barrack-room or hut. This arrangement may have an important bearing on the transmission of the infection from season to season.

First Outbreak, 1915. The Dépôt became severely crowded in November 1914, and this overcrowding was followed by a sharp outbreak in January and February 1915 ; 19 cases with 11 deaths occurred, followed by 10 cases with 6 deaths in the reserve battalions fed by recruits from the Dépôt. No information as to contact carrier rates is available, and no non-contact work was attempted. Nothing certain is known of the type of the disease, but it is probable that the majority of the cases were due to Type I meningococcus. No definite record of the overcrowding is available, but it was very severe.

Second Outbreak, 1916. The second outbreak began in February 1916. Seven cases occurred, all due to meningococcus Type II. Only contacts were examined, but this was done on a very wide scale by Captain M. Flack, and in most cases the ' contact ' carrier rate was over 20 per cent. I have no information as to the overcrowding, but as the ' mobilization ' standard of each hut was taken as 32 instead of 30, it follows that each man had only 37·5 sq. ft. of floor space, whilst, allowing for the stove, there would be almost exactly one ft. between the beds. It is, therefore, certain that there was some overcrowding in 1916, though I am informed it was not nearly so severe as in the preceding or following years.

All the carriers detected carried Type II meningococcus, a striking correspondence with the Type II found in all the cases.

Beginning of Non-contact Examinations. In August 1916 a large non-contact sample was taken by Captain Flack by swabbing 121 men who had been three months in the dépôt. He found a carrier rate of 10 per cent., an unexpectedly high figure, especially for the time of year.

He therefore decided to keep a continuous watch upon the carrier rate by means of large weekly swabbings, as he anticipated a return of the disease in the following winter. This watch was thenceforward continued up to June 1919.

The aggregate swabbings in September showed a carrier rate of 5 per cent., in October 6 per cent., and in November 4·6 per cent. So far all seemed well.

The dépôt was, however, rapidly filling up, as a result of the operation of the National Service Acts, and in the first week of November was for the first time above its ' mobilization ' accommodation.

On December 4 a number of Aylwin huts were occupied for the first time. On December 9 the overcrowding was at its zenith, over a thousand men more than the ordinary ' mobilization ' strength and 600 more than the ' extra mobilization ' strength being accommodated in drill sheds, &c., as well as the Aylwin huts.

The Warning Rise in December 1916. German measles and influenza now appeared, and the carrier rate, which had kept beautifully low during November (5 per cent. on November 29), now commenced to rise in ominous fashion ; on December 6 it was nearly 17 per cent., on the 21st, 19 per cent., just under the 20 per cent. danger line as laid down in the War Office memorandum.

Details of the warning rise are shown in the following table (note the increasing prevalence of Type II, to which type nearly all the actual cases were due). The close resemblance between the overcrowding curve and the carrier-rate curve which follows it two to three weeks after is shown on Chart V.

Date.	*Non-contact sample or contact.*	*Number swabbed.*	*Number positive.*	*Carrier rate (%).*	*Types*			
					I.	*II.*	*III.*	*IV.*
Sept. 7	Sample	127	10	8	3	3	..	4
,, 14	,,	123	1	0·75	..	1	..	..
,, 25	,,	119	7	6	..	5	2	..
Oct. 2	,,	120	5	4	1	3	1	..
,, 9	,,	139	9	6·5	4	4	1	..
,, 16	,,	118	9	7·5	4	4	1	..
,, 23	,,	117	7	6	2	4	1	..
,, 30	,,	121	6	5	..	3	3	..
Nov. 5	,,	90	3	3·25	2	..	1	..
,, 13	,,	115	2	1·75	2	..	..	..
,, 22	,,	113	9	8	3	1	5	..
,, 29	,,	120	6	5	2	3	1	..
Dec. 6	,,	120	20	16·75	2	17	..	1
,, 13	,,	119	14	12	12	2	..	..
,, 21	,,	125	24	19·25	3	20	1	..
,, 29	,,	91	10	11	1	9	..	..

Third Outbreak, 1917. The storm signal had been read aright, and at the end of December 1916 all the requisite factors for an epidemic of cerebrospinal fever were present, cold weather, severe overcrowding, a high carrier rate, and a population rendered susceptible by youth, by strange surroundings and methods of life, and by the depressing effects of nostalgia combined with the malaise of antityphoid inoculations and vaccination performed before acclimatization to their new circumstances was established.

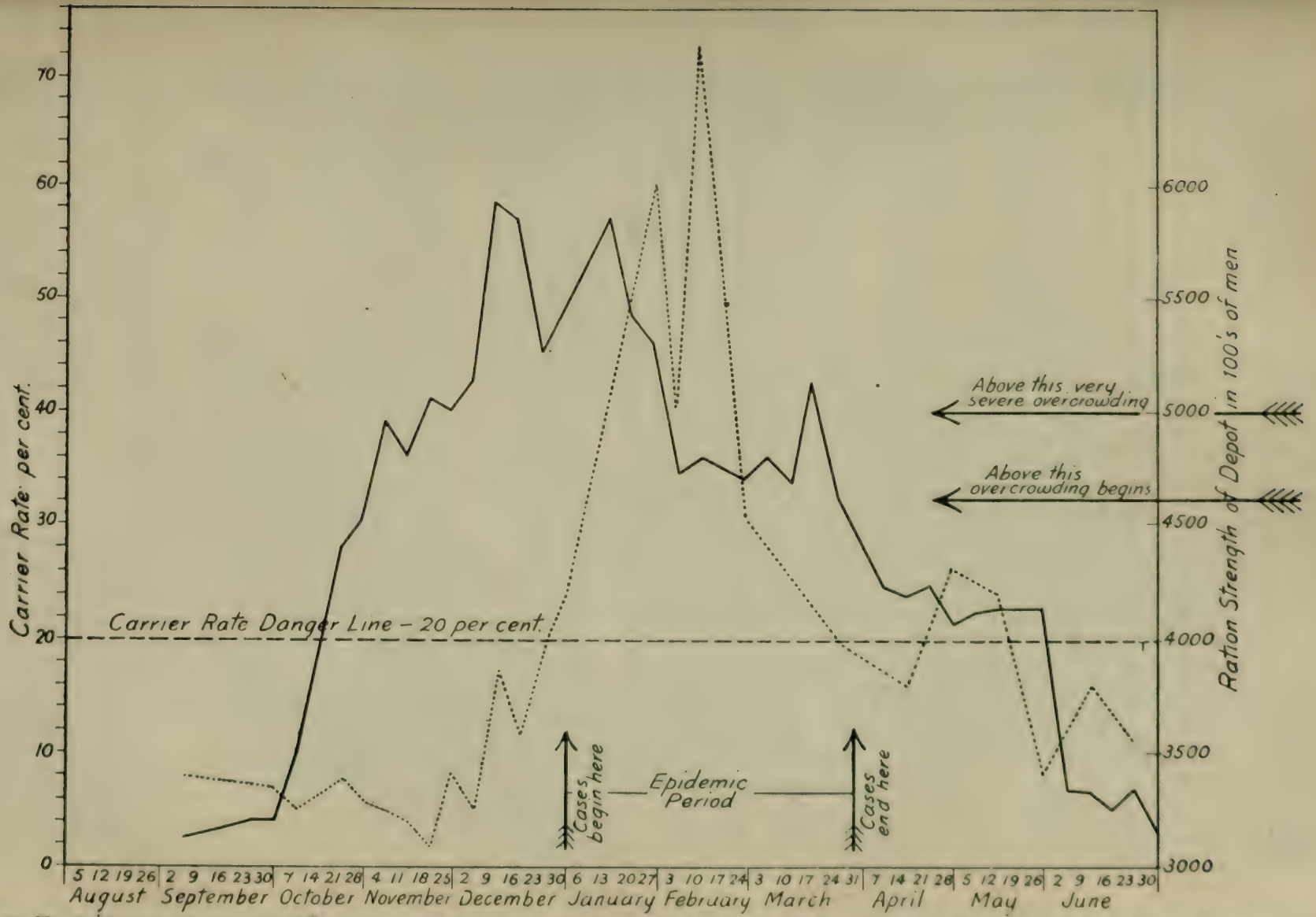


CHART V.—Influence of overcrowding upon carrier rate. Caterham Dépôt, 1917, third outbreak. Continuous black line = Ration strength in 100's of Dépôt (overcrowding begins at 4,600 and becomes severe at 5,000). Dotted line = Carrier rate per cent.

The first case was diagnosed on December 29, and was almost immediately followed by one other. The contacts (73) of these two cases showed carrier rates of 23 and 27 per cent. respectively. Both were due to Type II meningococcus, 17 out of 20 positive contacts carrying the same type.

No further cases occurred for three weeks, and owing to the unfortunate illness of Captain Flack, no samples of non-contacts were taken during this period. Captain W. Allan then took over the work for a few weeks, until he also became ill; his observations are those recorded from January 24 to February 19 inclusive.

During the same three weeks the Aylwin huts, which had been leaking badly, were given up, and 500 men were sent away from the dépôt to ease the overcrowding. Influenza of a mild type and bronchial catarrhs became increasingly prevalent.

Between January 24 and February 2 seven cases occurred in succession, three of them being fulminating cases, the patients dying within 24 hours of the onset. Of these cases, 185 contacts were swabbed showing a carrier rate of 46 per cent.

The first of these cases was due to Type I meningococcus, the rest were all due to Type II.

The contacts of the first case showed a carrier rate of 43 per cent., those of the second reached the appalling rate of 71 per cent., the hut containing 32 men, one of whom was the patient (Type II), ten of whom were carriers of Type I, eleven of Type II, one of Type III. Six of the nine remaining had organisms resembling meningococci which failed to agglutinate with the standard sera; only three were above suspicion.

A sample of non-contacts taken on February 5 showed the even higher rate of 72·5 per cent., the trained soldiers being almost as heavily infected as the recruits. The period of this group of cases exactly coincides with the great frost—the severest weather experienced in England since 1895.

It was now obvious that a severe epidemic was threatened, and the question of swabbing the whole garrison and isolating all carriers was discussed and negatived on account of the magnitude of the work, and the practical certainty that half the population was carrying.

Instead, the following measures were taken: parades were shortened, inoculation and vaccination stopped, men were spread out as much as possible (numbers were now slightly below 'mobilization' scale of accommodation), a certain number of windows in each hut and room were fixed open, and the crowding of Y.M.C.A.'s, &c., was limited.

The whole population of the dépôt received two courses of treatment with the Levick spray, using zinc sulphate solution 1 per cent.

Nine cases occurred in March.

The dépôt was reduced to normal mobilization accommodation in mid-February, but men were still 32 to a hut.

Twenty-one cases in all occurred in 1917 at the dépôt, and the great majority of both patients and carriers (nearly 70 per cent, 181 out of 260) were infected with the Type II meningococcus. Further details of this year's (1917) outbreak are to be found in *R.A.M.C. Journal*, January 1918, and *Journal of Hygiene*, July 1918.

Prophylactic Scheme, 1917–18. The carrier rate in August 1917 dropped to 2 per cent. This was evidently the acceptable time to try to prevent by early measures a return for the fourth time of the disease in the coming winter. In September 1917, a scheme of prophylaxis was submitted to the D.D.M.S. after consultation with the commandant and the senior medical officer of the dépôt. Its chief provision was the spacing out of all beds to 2½ ft. distance. This meant reducing the number of men in each hut from 32 to 23, thereby incidentally increasing each man's floor space from 37½ sq. ft. to 52 ; similarly it meant reducing the number of men in barrack-rooms from 36 to 26. This involved a reduction of 1,266 beds below the mobilization standard, the beds being returned to store.

The other provisions included simple devices for fixed (hopper) window ventilation, the postponement of anti-typhoid inoculation until the recruit's second month, provision of larger medical inspection premises, prevention of overcrowding in Y.M.C.A. and similar premises, and the provision of a large spray hut capable of treating two platoons simultaneously if necessary, and weekly samples of 100 men as a guide to the current carrier rate.

I have no doubt that the spacing out was by far the most important, ventilation coming second, and probably the postponement of the inoculation (until the recruit is to some extent acclimatized) third. Spray treatment was used for entrant recruits only until May.

The scheme was to come into force if a warning rise occurred in the carrier rate, or at the beginning of the ' danger period ', that is, the winter months. The carrier rate, which had been low in August 1917, showed a considerable rise in September, and on September 27 a sample of 100 trained soldiers (who are distributed two in each hut) gave a carrier rate of 17 per cent. Permission was then obtained to put the scheme into force, and ' spacing out ' was practically accomplished by October 4, 1917.

Success of Prophylaxis. The success of the scheme exceeded all expectations, the carrier rate fell steadily throughout the winter. The average for December 1917 was 3 per cent., compared with 16 per cent. average for 1916. For January 1918 it was 4 per cent. compared with 60 per cent. for January 1917. For February 1918 the average was 4 per cent. compared with the 72 maximum and 40 per cent. average for the February of 1916. For March 1918 the average was 0·5 per cent., compared with 20 per cent. for March 1917. This is only a quarter of what is usually regarded as the irreducible minimum of the civil population 2 per cent.

For the first time in four years of war the winter months had passed, not only without an outbreak, but without a single case of cerebrospinal fever. Not only had the scheme done what was intended in the prevention of cases of cerebrospinal fever and the elimination of carriers, but the dépôt had also been almost free from other infectious diseases, and had been extraordinarily healthy. That this was not entirely due to a favourable winter is shown not only by subsequent events, but also by the fact that another dépôt situated ten miles away, which was severely overcrowded, had a sharp outbreak of cerebrospinal fever during the January and February of this year. But perhaps the most striking proof of the efficacy of spacing out is

furnished by the fact that when overcrowding again began, and cases and high carrier rates followed in its wake, the infection was due to a type of meningococcus other than Type II to which had been due all the cases in 1916 as well as six-sevenths of the cases in 1917, and 70 per cent. of the carriers, as well as nearly all the cases in several subsidiary outbreaks in the reserve battalions fed by recruits from the dépôt. The house was, indeed, swept and garnished of its Type II infection, and when the overcrowding came again the devils, worse than the first that entered in and abode there, were Types I and III.

Resumption of Overcrowding, April 1918. Spacing out came to an end on April 19, 1918, after a reign of 198 days. Then came the great call up of previously exempted men, mostly miners. The dépôt authorities, particularly the senior medical officer, were fully alive to the danger of overcrowding, and from the first made strenuous efforts to prevent it, but so fast did recruits pour in that extra accommodation in the shape of tents toiled painfully in the rear of the rapidly increasing strength of the garrison instead of anticipating it. Hundreds were continually arriving without notice and had to be put in already overcrowded accommodation.

Overcrowding began on April 20, and by April 25 it was extremely severe, worse than at any previous time, remaining very severe for three weeks. The carrier rate had been 1 per cent. on April 10. On April 17 and 26 new arrivals only were examined, those on April 26 not having slept more than one night in the dépôt, showed 3 per cent.

Fourth Outbreak, 1918. A sample of 100 men on May 2, who had been some time in the dépôt, showed 34 per cent. carriers.

The warning rise had, indeed, come again. Three to 34 per cent. was unmistakably ominous. Agglutinations were complete by May 5, and the carrier rate duly reported. The season was May, and it was hoped that the danger season was so far past that the omen would be nullified.

On May 9 the Advisory Sanitary Council were at Caterham, and the first case was diagnosed by lumbar puncture. Thus, by the aid of the warning rise, the epidemic was foreseen and aborted almost at once. Not that common sense could not have foreseen the danger of such extreme and severe overcrowding, but by the aid of the warning rise, it was possible to fulfil the canon of Hippocrates (*Epidemics*, Book I. p. 5) [1] with regard to this outbreak as perhaps it had never been fulfilled before.

One barrack-room on May 9 showed the high carrier rate of 41 per cent. On May 19 and the three following days four cases occurred, all, like the first, due to Type I meningococcus. On and after May 20 all men (about 4,000) who had been three weeks or more in garrison received a six days' course of spray treatment with a solution of zinc sulphate, 1·2 per cent., in the spray hut, each man having ten minutes' treatment each day. A large sample of 375 of these men examined immediately after the conclusion of the six days' treatment showed an average carrier rate of 16 per cent., those from barrack-rooms being still well above 20 per cent. By May 25,

[1] 'The physician must be able to tell the antecedents, know the present, and foretell the future—must meditate these things and have two special objects in view with regard to disease, namely, to do good, or not to do harm.' *Epidemics*, Bk. I, p. 5.

however, the accommodation allowed of a ' mobilization ' standard, and by June 8 tentage had been obtained sufficient to allow of the following modified spacing out : 7 men to each tent, 27 to each hut, and 30 to each barrack-room.

These figures allow less than 2 ft. between the beds in huts and rooms. For some weeks longer the carrier rates of the barrack-rooms especially remained high despite the fine weather, and taking only barrack-room carrier rates

20 per cent. was found on June 19 ;
16 per cent. on July 10 ; and
19 per cent. on July 17.

Now ensued a period of great anxiety. There were over 13,000 men in the dépôt, the bulk of them (some 8,000) in tents, 7 in a tent, with an average carrier rate of about 10 per cent. ; about 3,900 in huts, 27 to a hut, with an average carrier rate of 8 per cent. (the huts had not been overcrowded in May), and about 1,000 in barrack-rooms (30 to a room) with higher carrier rates on an average of, say, 15 per cent.

Such then was the state of affairs when the first great wave of influenza passed through the dépôt in July 1918. Nearly 4,000 cases of influenza occurred and 15 deaths. It is a great matter for congratulation that the overcrowding had been abated by June 7, that is, several weeks before the epidemic occurred, and it may be regarded as practically certain that the small number (seven) of men in each tent, and the comparatively well-spaced-out huts with their low carrier rates prevented another outbreak of cerebrospinal fever following the influenza. As it was, only two cases occurred.

One case was certainly due to Type II meningococcus and the other probably so.

No further cases of cerebrospinal fever occurred, and with the decrease in the population in August due to large drafts, the carrier rates came down satisfactorily, reaching 0 per cent. in mid-October.

A case of tuberculous meningitis occurring in a Q.M.A.A.C. girl working in the dépôt, all Q.M.A.A.C personnel in the dépôt were examined, and three carriers (2 II, 1 I) were found in the 83 girls examined. This is practically the normal civil rate. The sleeping quarters were good and there was no overcrowding. This was the first large non-contact sample of women that I had taken.

Soon after this came the second wave of influenza, but although this was followed by a certain rise in carrier rates (see Chart VI) these only reached 12 per cent., and no cases of cerebrospinal fever occurred.

An examination of selected patients in the Caterham Hospital was carried out on October 25. Men with acute influenza in an early stage, or with influenzal pneumonia were examined. Four carriers were found in 27 ; a proportion not unduly high.

On the same day men with slight pyrexias in the detention hut were examined, no carrier being found in nine men. The infection was certainly in no way due to the meningococcus.

By November 6 the second influenza epidemic was obviously abating, the ration strength was down to 3,275, and the carrier rate was 8 per cent.

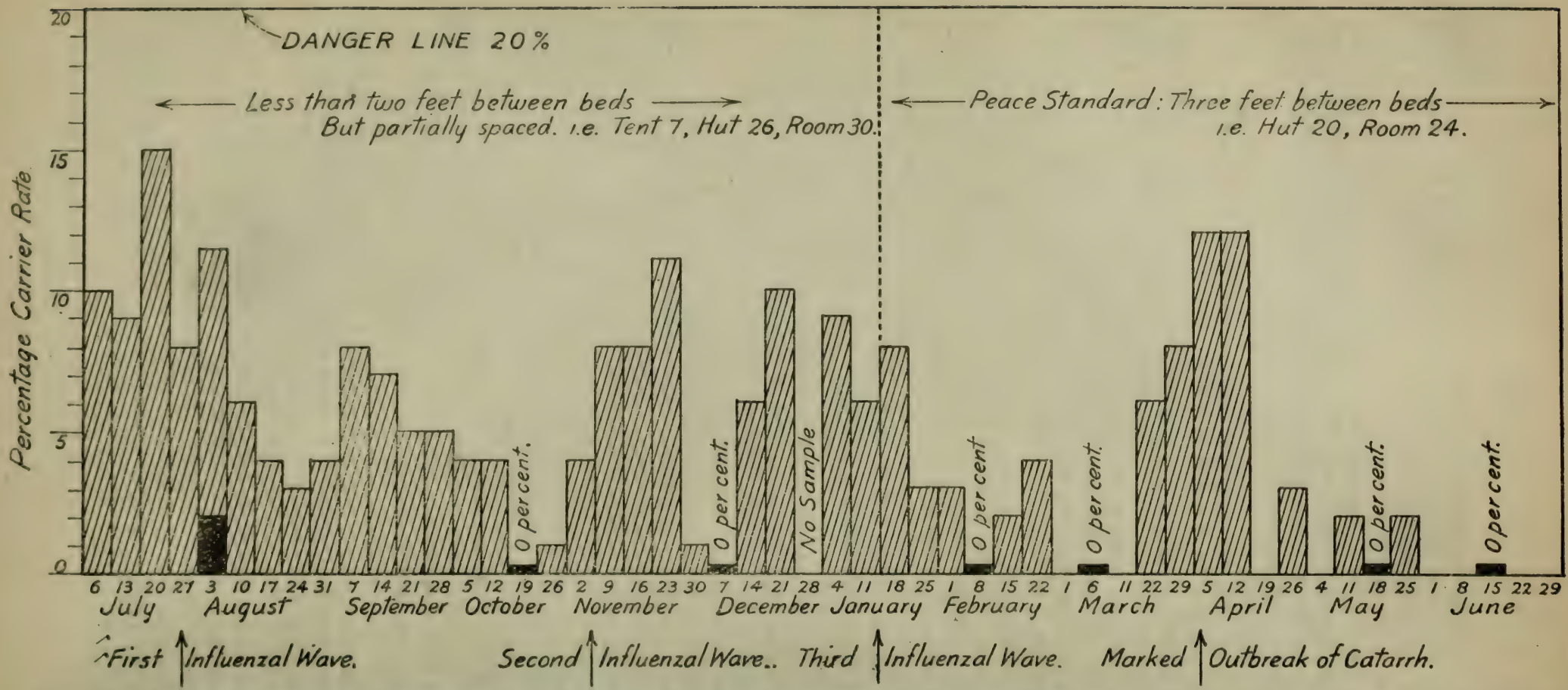


Chart VI. Caterham. Non-contact carrier rates during the twelve months July 1918 to June 1919, showing the effect upon the carrier rate of the three waves of influenza.

Note.—Overcrowding had been ended about three weeks before June 7, 1918.

Two cases of cerebrospinal fever occurred after the first wave in the first week of August.

The next date of importance is January 9, as on this date orders were given that the dépôt should be spaced out to the 'peace' standard (60 sq. ft., 600 cubic ft., 3 ft. between beds) of 24 men per barrack-room, and 20 per hut, which standard had been in abeyance since October 1914.

The third great wave of influenza disturbed the 'peace standard' dépôt but little, and, except for another slight rise in the carrier rate to 12 per cent., no meningococcal activity was noticeable. A hospital sample of patients with catarrhs and influenza soon after gave the same carrier rate 12 per cent. Even freshly admitted recruits (second and third day) gave 8 per cent., so it is possible that the general civil carrier rate was somewhat higher than usual in March and April, 1919, owing to the previous influenzal waves.

After the middle of April, carrier rates faded away to the 'normal' 2 per cent. of the civil population or even to nothing.

During this danger period the spray hut had been kept in readiness, but no occasion arose for its use. No isolation of carriers was practised, except in the case of the few actual positive contacts of the two patients. Carriers of epidemic strains of meningococcus (agglutinable) and 'pure plate' carriers of non-epidemic (inagglutinable) strains of organisms resembling meningococci were periodically re-examined. A few found to be chronic were given a week's course of treatment with flavine (without isolation).

In a properly spaced out and freely ventilated barrack, the isolation of carriers detected in non-contact samples is unnecessary, though it is probably desirable to isolate positive contacts of actual cases in the hope of isolating the actual causal or infecting carrier, who may be presumed to be carrying a virulent strain. The risk of cases occurring in a barrack with peace standard accommodation appears very small.

To illustrate the preceding summary Chart VII has been drawn, showing the non-contact carrier rates for the three years during which continuous observation was kept (by weekly sample swabbings of 100 men), together with the occurrence of overcrowding and of cases of the disease. In the chart it is evident that overcrowding is the main cause alike of high carrier rates and of cases, and that season is a factor of only secondary importance. A comparison of the two twelve-month periods July 1916 to June 1917 and July 1917 to June 1918, as shown upon Chart VII, is especially interesting. Here we have two periods of severe overcrowding separated by a period of rather more than a year. During nearly half of this interval, which half includes the whole of what is usually considered the danger season of cerebrospinal fever, beds are rigidly 'spaced out' to a distance of 2½ ft. between each (in a Guards unit these distances are correctly kept). During this latter six months there are no cases and carrier rates sink to nothing.

Moreover, 'spacing out' almost completely purges the dépôt of its previous Type II infection.

The second period of overcrowding brings its own infection, a Type I type.

The second period of overcrowding did not force the carrier rate so high as the first, but this was probably solely due to the fact

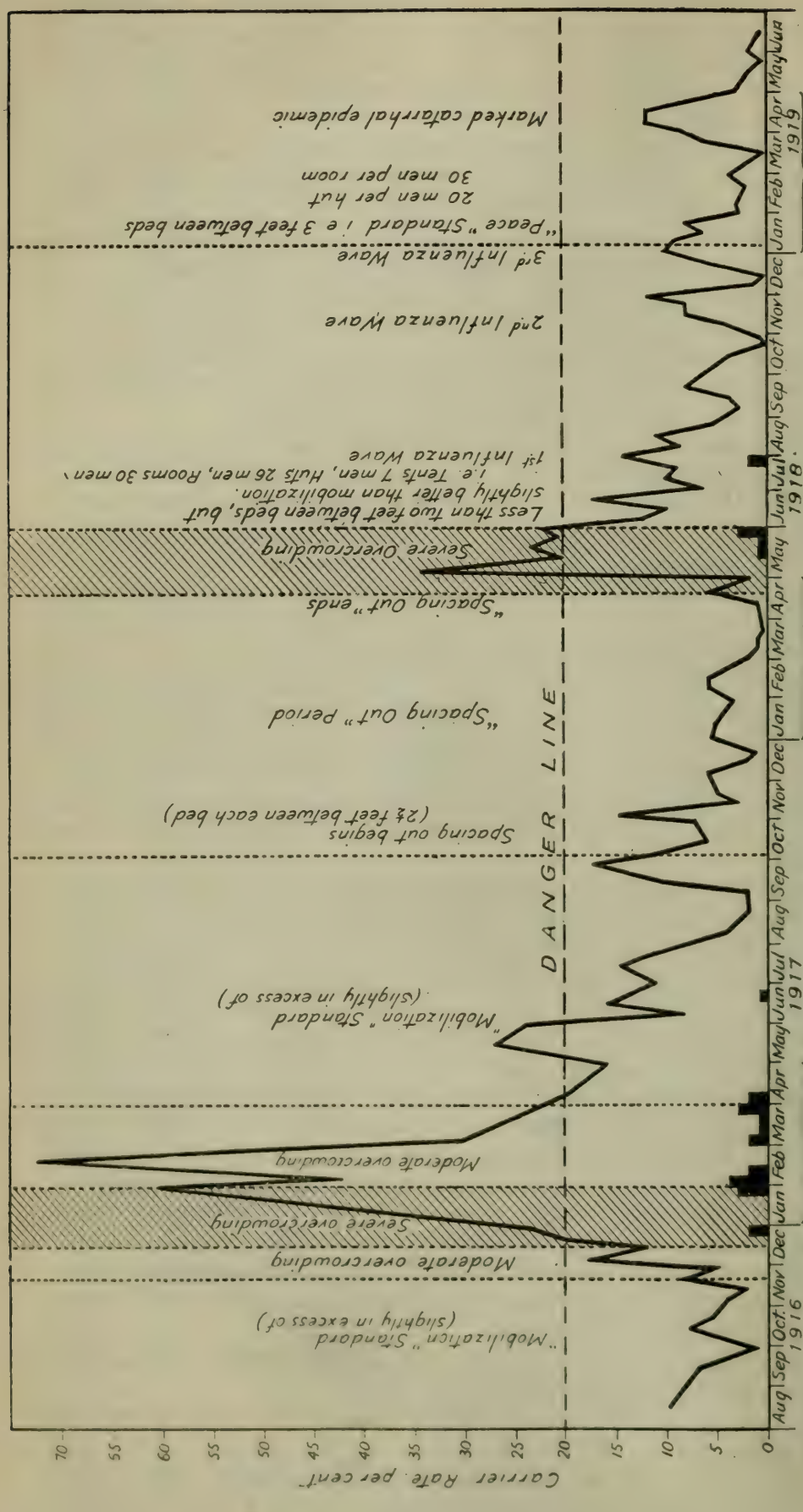


Chart VII. Caterham Dépôt. Non-contact carrier rates for three years, estimated weekly by sample swabbings of 100 men (only carrier of serologically qualified (epidemic) meningococci are included), shown by heavy black line.

Small black columns represent the number of cases of C.S.F. occurring in a week, e. g. last week of December 1916 two cases, last week of January 1917 three cases, first week of February 1917 four cases, &c.

Shading = periods of *severe* overcrowding.

that the overcrowding (whilst quite as severe) did not last nearly so long. Nevertheless, each period of overcrowding produced an extremely well-marked wave in the non-contact carrier rate, which rose sharply to the 20 per cent. danger line before the occurrence of cases.

The rise in the carrier rate becomes noticeable about 14 days after the commencement of severe overcrowding, cases beginning about a week after the 20 per cent. line is reached.

A marked rise in the proportion of carriers of epidemic strains (that is, those agglutinating with the four standard serums of Gordon) to carriers of organisms culturally resembling the meningococcus, but not so agglutinating, was also found in the non-contact samples after the commencement of each period of overcrowding. This, we have seen, may be considered as constituting a second danger signal.

Cases in each case ceased as soon as the overcrowding was abated, carrier rates coming down much more slowly than they went up.

That the overcrowding was cut short so promptly on the second occasion was due largely to the bacteriological evidence of danger revealed by the sample of May 2, and it is gratifying that only five cases occurred when it is remembered that the population at risk was considerably more than twice as great as during the previous outbreak.

On both occasions it was shown that recruits were entering with a low carrier rate which multiplied rapidly in the overcrowded state of the garrison.

The average non-contact carrier rate was substantially the same as the average contact carrier rate of the same period for the same class of accommodation. Thus, during the epidemic period of 1917, that is, from December 29, 1916, to March 31, 1917, 426 contacts gave 145 carriers (38 Type I, 103 Type II, 2 Type III, 2 Type IV), a carrier rate of 34 per cent.

Whilst, during the same period, 336 non-contact samples (from the same type of accommodation) yielded 115 carriers (31 Type I, 78 Type II, 2 Type II, 4 Type IV), also a carrier rate of 34 per cent.

During the second outbreak, the number of contacts is too small for comparison of much value, whilst some of the non-contacts had not been sufficiently long exposed to the overcrowding ; 51 contacts gave 10 carriers (5 Type I, 3 Type II, 2 Type III), a carrier rate of 20 per cent.

Whilst, during the same month, 1,132 non-contacts yielded 174 carriers (78 Type I, 54 Type II, 41 Type III, 1 Type IV), a carrier rate of 15 per cent.

H. *Correspondence of Prevalent Carrier Type with Prevalent Case Type.*

The correspondence of the prevalent type of case with the prevalent type of carrier demonstrated in the Chatham outbreak (Armstrong and Tulloch) is remarkably well shown at Caterham.

Thus, in 1916, all seven cases were due to Type II and all 56

carriers detected amongst the 431 contacts of these cases (contact being here interpreted to include contacts of contacts) were also carrying Type II.

In 1917, during the period of the epidemic, there were found 69 carriers of Type I to 181 carriers of Type II, 4 Type III, 6 Type IV, that is nearly three times as many carriers of Type II as of Type I. The cases were distributed thus, 2 due to Type I, 13 due to Type II, 6 not typed. During the whole year, July 1916 to June 1917, the carrier figures are in much the same proportion, 126 carrying Type I, 336 carrying Type II, 30 carrying Type III, 12 carrying Type IV.

In 1918, during the epidemic period (May) all five cases were due to Type I and the carriers were, as we have seen, 83 Type I, 57 Type II, 43 Type III, 1 Type IV.

Nearly all the 43 Type III carriers had much group agglutinin for Type I.

The vernal renaissance of Type II appears an annual event, having been noticed to some extent in 1917, 1918, and 1919.

In the last year of the war, i. e. from July 1, 1918 to June 30, 1919, there have been two cases due to Caterham infection, one due to Type II and one untyped but probably due to Type II, whilst of the 172 carriers found throughout the year, 87 have been Type I, 62 Type II, 7 Type III, 16 Type IV.

Diagram 7 shows the variations in carrier rate during this year, mostly consequent upon the three waves of influenza.

Note on Correspondence of Prevalent Carrier Type (in non-contact samples) with Prevalent Case Type.

This correspondence is shown to be just as well marked if we compare the types of all non-contact carriers found in any year in the London Command with the types of the cases occurring in that year in the London Command, irrespective of the actual places where the samples were taken or the cases occurred.

Thus:

I. Total Non-Contact. Examinations in three consecutive years in London Command.

Twelve months period.	*Number examined.*	*No. of carriers found.*	*Percentage carrier rate.*	*No. of carriers of each type.*			
				I.	*II.*	*III.*	*IV.*
Ending June 30, 1917 .	3,122	399	12·7	90	265	34	10
Ending June 30, 1918 .	8,675	812	9·3	389	308	96	19
Ending June 30, 1919 .	4,209	243	5·8	122	81	19	21

II. Total cases in London Command same years.

Twelve months period.	*No. of cases.*	*No. of cases in which Type is not determined.*	*No. of cases due in each Type.*			
			I.	*II.*	*III.*	*IV.*
Ending June 30, 1917 .	71	19	19	24	7	2
Ending June 30, 1918 .	21	2	9	7	3	0
Ending June 30, 1919 .	22	2	11	6	3	0

III. Comparison percentage of carriers of each type to total number of carriers (roman) and percentage of cases due to each type

to total number of cases (*italic*) of KNOWN TYPE. Non-contact carriers, roman; case, *italic*.

Twelve months period.	*Type I.*	*Type II.*	*Type III.*	*Type IV.*
Ending June 30, 1917 [1] . .	22 : *36*	66 : *46*	8 : *13*	2·5 : *4*
Ending June 30, 1918 . .	48 : *47*	38 : *37*	11 : *16*	2 : *0*
Ending June 30, 1919 . .	50 : *55*	33 : *30*	8 : *15*	8·6 : *0*

[1] In this period in 19 out of 71 cases the type was not determined: most of these 19 were probably Type II.

It may be interesting to sum up the known cases of cerebrospinal fever, traceable to Caterham infection, during the war.

	Caterham Dépôt.		*Other Camps and Barracks receiving Recruits from Caterham.*	
	Cases.	*Deaths.*	*Cases.*	*Deaths.*
1914 (half year) .	0	0	0	0
1915 . . .	19	11	10	6
1916 . . .	7	0	0	0
1917 . . .	21	7	6	4
1918 . . .	7	2	3	0
1919 (half year) .	0	0	0	0
Totals . .	54	20	19	10

Total military cases: 73 cases with 30 deaths.

Four civilian cases attributable to this source have also been investigated.

It must also be remembered that the population at risk in 1918 was more than twice that of any other year, though the overcrowding occurred at a much more favourable season of the year.

The chronicles of Caterham cannot be concluded without a reference to the great kindness of the late Commandant, Lieut.-Colonel Sir William Ingleby, and of the Senior Medical Officer, Brevet-Surgeon Colonel W. H. Whiston, Irish Guards, who have, often at grave inconvenience, made every arrangement and allowed every facility for the work (which must have caused much derangement of other programmes) and who have met every suggestion with the greatest readiness and cordiality.

I. *Higher Carrier Rate and Mass Infection.*

Whilst sporadic cases may admittedly arise with any carrier rate, can we assign any reason why a high carrier rate (i. e. over 20 per cent.) should appear so usual a concomitant of anything like an epidemic? That it is so, has been reported from regions so far remote as Hong Kong, where Olitsky, investigating the recent epidemic, took a large non-contact sample in the jail, and found a carrier rate of 25 per cent., corresponding in type to the case type of the epidemic (Type II).

One reason is the obvious one, that the higher the carrier rate the greater the chance of the susceptible sleeping next to a carrier.

The second is that a high carrier rate means overcrowding, and overcrowding, in addition to high carrier rate, means lowered resistance to cerebrospinal fever as indeed to every other disease.

But this is not all; we have seen in Diagrams III and IV that as the carrier rate rises, so does the proportion of epidemic meningococci agglutinating standard type serums, and it has also appeared

throughout the long series of experiments that the higher the carrier rate the more abundant and the more nearly pure are the cultures of meningococcus obtained.

In other words, when the plates upon which the secretion from the swabs of a sample with a high carrier rate have been inoculated are inspected after 24 hours' incubation, if the carrier rate is (after subculture and agglutination) to prove a high one, many of the growths apparent on first culture are of profuse and of almost unmixed meningococcus.

A really bad sample is, after 24 hours' growth, unmistakable to an eye experienced in the medium used, and is a striking demonstration.

A high carrier rate, therefore, means that not only is the number of carriers increased but also that most of these carriers are ' pure plate ' carriers such as we often find in the investigation of cases where a soldier-father on leave appears to have infected his child.

These are probably the most dangerous class of carrier.

Assume then a community in which the carrier rate is 33 per cent. Every third man is not only a carrier, but practically every third man is a carrier of the abundant ' pure plate ' dangerous type. Add to increase the carrier's range and frequency of discharge the explosive sneeze of an influenza, which has also lowered the resistance of the susceptible recruit, already temporarily decreased by recent inoculation and vaccinia, by the new hardship and by nostalgia, and we have the exact conditions requisite for an outbreak. When, too, the fact is appreciated that at Caterham at least 100 recruits carried to every one who developed the disease, it is apparent that the average resistance to meningeal invasion is very high, and to overcome it the massive dosage ensured by such a mass infection as we have described, may be necessary.

The beneficial effect in checking a case epidemic sometimes following the use of the zinc sulphate spray may be due to its possible effect of reducing this mass infection.

J. *The Rapid Reduction of Carrier Rate by increasing the distance between Beds.*

During the period of nearly all these investigations the attainment of the peace standard of 3 ft. between beds was almost impossible owing to the pressure upon accommodation.

Full mobilization standard meant in most barrack-rooms and huts a distance between beds of 1 ft. 4 in., and this, even rigidly carried out, was not sufficient to produce a rapid improvement in an already high carrier rate.

As at Caterham, so in the other units, a purely arbitrary standard midway between was therefore selected, and the beds being 2½ ft. across, 2½ ft. was selected as the space between. In a 60 ft. by 20 ft. hut this allows 23 men (or 24 if the stove be not working) to sleep without the side of any bed being less than 2½ ft. from that of its neighbours, and each man has approximately 53 sq. ft. of floor space (with 23 men).

In a 60 ft. by 15 ft. hut, mobilization standard, if strictly enforced,

only allows 22 men, so that with such a hut there is no difficulty in attaining the desired distance.

A similar provisional standard was attempted with barrack-rooms, and in every case attempts were also made to increase the ventilation by the fixation of windows, some being converted by wooden sides to fixed hopper ventilators.

Aylwin huts were reduced from eight men to five. Loose boxes which had held four men were spaced out to three, allowing wide separation of the heads, and giving 40 sq. ft. per man instead of 30.

The effects were exceedingly gratifying and occurred in all classes of accommodation (excepting one or two where ventilation was absolutely defective). Amongst the most striking were the barrack-rooms of four units which were severely overcrowded (see Chart VIII).

Effects of Spacing Out on the Carrier Rate in severely overcrowded Barrack-rooms.

Carrier rate per cent. before spacing out.	*Weeks spaced out.*	*Carrier rate per cent. after spacing out.*
22	8	2
28	6	7
38·5	6	4·5
28	5	4·5

K. *Carrier Rates in Aylwin Huts.*

Observations on men in Aylwin huts belonging to the same unit as the second barrack-room shown in the Diagram VII, form an interesting control experiment in two ways.

They remained overcrowded (eight men per hut) all through the same six weeks during which the barrack-rooms were first spaced out and in which the barrack-room population showed a decrease of carrier rate from 28 to 7 per cent.

They showed a carrier rate of 12 per cent. at the beginning of this six weeks (October to mid-December), and one of 15 per cent. at the end: in other words, whilst the spaced out barrack-rooms improved so much that they had only a quarter of their first percentage of carriers, the Aylwin huts showed a percentage raised from 12 to 15.

These carrier rates are high and show a rise, but it is somewhat surprising that the figure was not higher as the floor-space with eight men in an Aylwin hut is only 26 sq. ft. and the cubic space only 170·5. These so-called huts are probably safer than they would appear, owing to the porous nature of their material and the numerous leaks, &c., through which copious draughts of fresh air continually pour. They really approximate much more to tents than huts.

Now, on analysing this 15 per cent., it was found that out of twelve huts three were free of carriers, four had only one carrier, whilst one hut had three carriers amongst its eight men and one had four, that is to say a carrier rate of 50 per cent. in this one hut, although the general carrier rate was only 15 per cent. This is an example of the fact that small subdivisions (such as these huts), whilst they to some extent limit infection, yet in another way focus it.

The chances of a susceptible individual contracting cerebrospinal fever if introduced into such a community as the last-mentioned hut, half of whom are carriers housed with 26 sq. ft. of floor space and 170·5 cubic ft. of air space per man, must be considerable in the

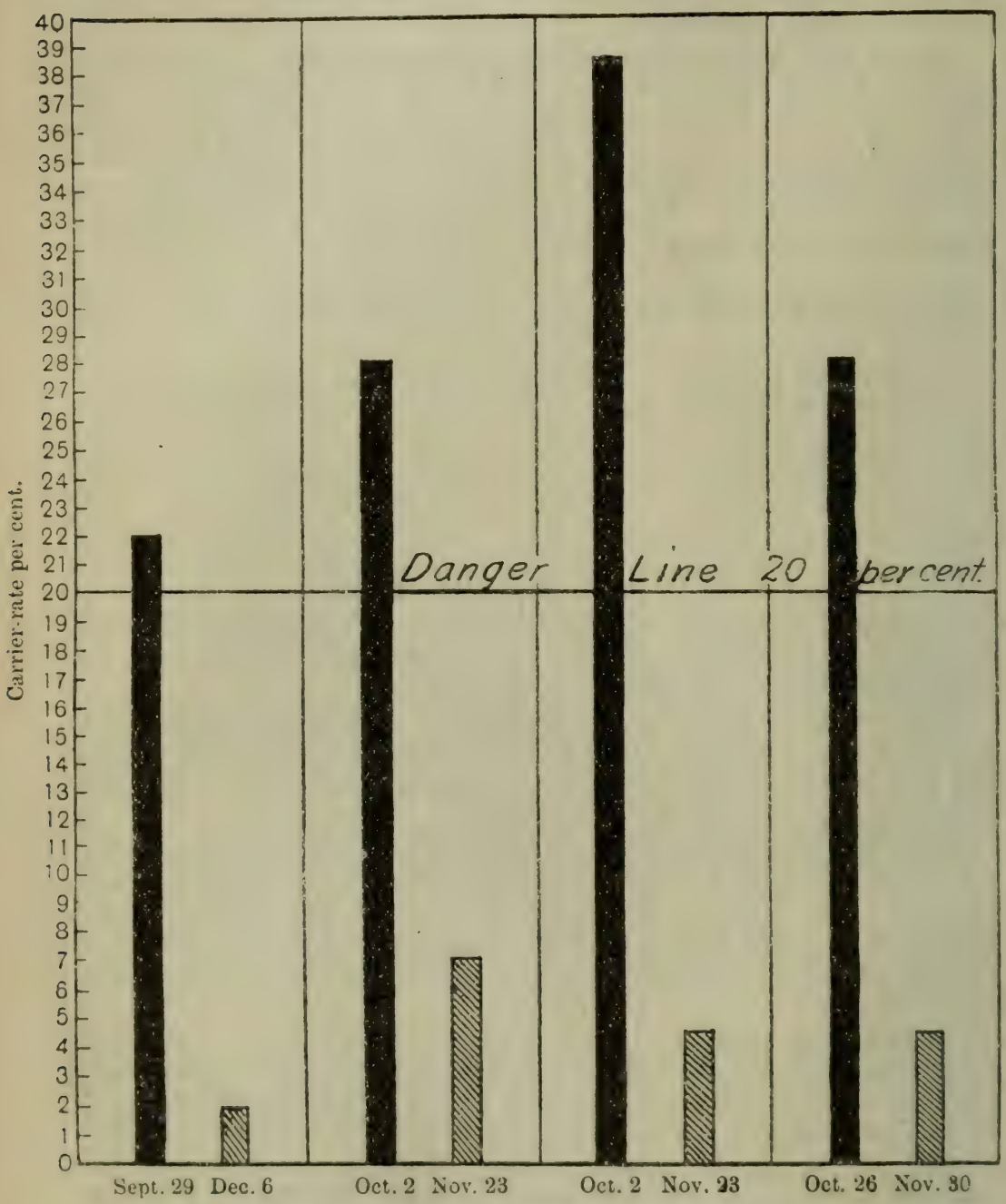


CHART VIII.—Rapid reduction of the carrier rate produced by spacing out in four severely overcrowded barrack-rooms. Carrier rates whilst severely overcrowded = black columns; carrier rates after 6–8 weeks' 'spacing out' = shaded columns. (All beds spaced out to 2½ ft. interval.)

month of December, and on the results being reported, the conditions were immediately remedied.

This was done by removing three men from each hut, leaving five men, who would, thus, each have 41 sq. ft. floor space and 272 cubic ft. air space.

It is impossible to say how far the beds were apart, as each Aylwin hut is a law unto itself.

After this arrangement had been in force five weeks, they were again examined and the carrier rate found to be 6 per cent. Moreover, no hut had more than one carrier in it.

L. *Tents.*

The number of men to be housed in a regulation bell tent is not strictly defined and has been a matter of some discussion. The official manual of elementary military hygiene calls a full complement 15 men, which allows 12 sq. ft. and 32 cubic ft. per man. The writer, when a private in the South African campaign, slept regularly as one of 16 men (occasionally 18) until tents were abandoned. The pressure of feet at the pole with 15 or 16 men is so great that the wearing of boots is almost essential to sleep.

Tent contact was of great importance in the transmission of enteric fever in pre-inoculation days, Lelean pointing out that in the Spanish American War 66 per cent. of cases were considered traceable to infection by other men in the same tent.

Some light has been shed on the problem of the safe number of men to place in each tent by the carrier rates found in the examination of samples from tented units.

This safe number would appear to be seven or eight, preferably seven, and in the London district for the last two years seven has been adhered to for recruits (who undoubtedly require special spacing).

Much depends upon the weather. In exceptionally fine weather, with tent flies rolled and doors open at night, conditions approximate to open bivouac with the advantage of much less dew. But it is very seldom that men will tolerate this in England. Assuming then that men sleep with feet to pole at regular intervals, 15 men in a tent allows just 2 ft. 4 in. between the centres of heads, or when two men are facing each other their mouths are not more than 1 ft. 8 in. apart, a condition obviously very favourable to infection of a respiratory nature. The circle of mouths will be found to have a radius from the pole not exceeding 5 ft. 6 in. With seven men in a tent, centres of heads are about 5 ft. apart and mouths about 4 ft. 4 in. apart, a condition approximating to the effect produced in barrack-rooms and huts when Army plank beds 2½ ft. across are at intervals of 2½ ft. as in the spacing out hitherto described.

The following illustration shows a unit previously infected somewhat heavily:

A detachment of 101 men were sent into camp to ease the pressure on an overcrowded barrack in London, in late September and in fine weather.

They undoubtedly left London with a high carrier rate. After a week under canvas all were swabbed.

The general carrier rate was 16 per cent.

Of the 101 men 60 slept in tents (bell single-flap) each holding eight men or less.

Of these the carrier rate was 11 per cent.

Forty-one men slept in four tents holding nine, ten, and eleven (two tents thus) each.

Of these the carrier rate was 22 per cent.

Now consider the distribution in tents: out of thirteen tents four were quite clear of carriers; four had only one carrier; three had two carriers; and two had three carriers.

In only one tent was there a mixture of carriers of different types; this tent had three carriers among nine men, two carrying Type II meningococcus and one carrying Type I meningococcus; all the other four tents which had more than one carrier bred true, that is, had them of the same type. This grouping is suggestive of infection taking place in the tents and not brought from London, as the London source had carriers of both types pretty equally divided. It may of course be fortuitous.

Thus we see in a unit with a general carrier rate of 16, but divided into small communities, the difference in risk to a susceptible individual may be immense according as he may happen to sleep in a tent free of carriers or in one in which three out of the nine occupants are carriers.

The highest carrier rate I have found in a tent was in one containing nine men. They had, however, been previously exposed to intense overcrowding and had been in the tent about a fortnight. One developed meningitis due to Type I and four of the remainder were found to be carriers of the same type, a carrier rate of 55 per cent.

This high carrier rate was due to two factors, the previous overcrowding and the fact that the patient who developed the meningitis had a severe and incessant cough during his incubation period when he was no doubt a carrier.

Nine men in a tent here, as in the previous example, did not give any chance of a high carrier rate diminishing rapidly.

M. *Emergency Prophylaxis consequent upon Determination of Carrier Rate.*

It is always impossible to say definitely that any measures averted an epidemic, but the following is an example of a probable instance.

A certain training battalion, composed very largely of new recruits, was quartered in a camp in Surrey entirely in 60 ft. by 15 ft. huts during November 1918.

It had during this month suffered severely in the second great wave of influenza.

Certain huts had been set aside for the segregation of influenza cases, not deemed sufficiently ill for removal to hospital, upon which there was enormous pressure at this time.

These huts had unfortunately been allowed to become overcrowded, and in addition men were being discharged from them still coughing and sneezing vigorously.

A boy of 18, a new recruit, had arrived on October 29, had received his first anti-typhoid inoculation on November 2, his vaccination on the 6th, and fell ill of influenza on the night of November 9–10. He was removed to an 'influenza' hut, where instead of the mobilization

number of 22 healthy men, there were on several nights 30 influenza patients. On the 18th, having had a normal temperature for several nights, he was discharged to his original hut once more. On the 19th evening he became again ill and violently delirious, was sent to hospital on November 20 as an incipient pneumonia. On arrival he was immediately recognized as cerebrospinal fever, which proved to be due to Type II.

On the 21st all men remaining in the influenza hut (21), all men discharged from it during the time the patient had been in (19), and all men in the patient's original hut (23), totalling 63 in all, were examined.

On the afternoon of the 22nd the plates were examined, and a rapid rule of thumb method of estimation (based on inspection only, but useful for such emergencies as this) showed an extremely high carrier rate.

The battalion was under orders to move to another camp in an adjacent county and in another command on November 24. Agglutination tests were being carried out, but the results could not be complete for at least two days. I had confidence in the rapid estimation method (which agglutination showed subsequently to be a slight under-estimate, the final carrier rate being 47) and asked permission of the Senior Sanitary Officer of the D.D.M.S. London District to communicate the results direct to the Senior Sanitary Officer of the command into which the battalion was due to move.

In consultation with him it was arranged that the battalion should move as arranged, only the actual contacts being left behind pending agglutination results.

Accommodation (huts 120 ft. by 20 ft.) was to be reserved for it, sufficient to give every man at least 6 ft. of wall space (i. e. = 60 sq. ft., in this pattern hut).

Actual positive contacts were isolated (30) by us, but no further isolation or treatment was practised. No further cases occurred and the carrier rate as determined by Capt. Dickson soon after their arrival had fallen to about 15 per cent.

Details of the results of the rapid method and agglutination tests are shown under.

Hut.	*No. of Men Examined.*	*Carrier Rate by Rapid Method.*	*Carrier Rate by Agglutination.*	*Total Positives by Agglutination.*	*Types by Agglutination.*	
					I.	*II–IV.*
Patient's original hut to which he returned .	23	48 per cent.	43 per cent.	10	1	9
'Influenza' hut men still remaining . . .	21	50 „	57 „	12	3	9
Men recently discharged from 'influenza' hut .	19	33 „	42 „	8	2	6
Totals . . .	63	43 „	47 „	30	6	24

The great preponderance of the II–IV carriers is interesting in that the patient's type of meningococcus was also II, with considerable group agglutination for Type IV. He slept next a 'pure plate' carrier both in his own hut and in the influenza hut.

Here, although of course we cannot say for certain, it is extremely

probable that prompt and effective spacing out to 6 ft. of wall space prevented an outbreak. Many menacing features were certainly present on November 21, overcrowding, precedent influenza, high carrier rate combined with a most susceptible population, mostly boys of 18, fresh from home and recently inoculated.

This spacing out was rendered possible by the prompt detection of the high carrier rate. Had agglutination results been waited for the battalion would have moved, and subsequent spacing out would certainly have been more difficult to arrange and valuable time might have been lost.

At the same time, of course, a great many men had already been infected nasopharyngeally, and why only one succumbed to meningingeal invasion is a mystery. He certainly had had a severe combination of resistance-lowering factors, but so had many others, and yet he only fell ill. It is obvious that we must not claim too much credit for the non-occurrence of other cases.

N. *Corners and Partitions.*

The frequency with which men sleeping in corners are found to be carriers is striking, and in winter beds near the stove are often occupied by carriers.

The first is due to the dead-end nature of the ventilation in corners, and is an argument against the use of divisions bisecting huts and rooms, which are sometimes seen and, it must be confessed, are liked by the occupants. The partition of course doubles the number of corners and thus the number of dead-ends ; it also has an effect on the spread of infection similar to that studied with the Aylwin huts : it limits but focuses infection.

Two examples may be quoted of the interesting effect of partitions in huts and rooms :

The first is a hut of the usual dimensions, 60 ft. by 20 ft., but of superior construction, and with larger windows of the French window type. It had a partition dividing it into two. In this hut which, on mobilization standard, should have accommodated 30 men, 42 men were sleeping, 18 in one side and 24 in the other, instead of 15 in each compartment.

Four of the 18 on one side of the partition were carriers, two sleeping side by side.

Of the 24 in the other compartment, only 22 were examined, and of these nine were carriers with no less than six Type I carriers in adjacent beds on one side of the hut. A clearer case of cross-infection could not be imagined.

It will be observed that the carrier rate of one-half is 22 per cent. ; that of the more severely overcrowded half is 37 per cent.

To show the possible influence of a partition in a room under fair conditions, and to demonstrate what can be found out sometimes by examining closely the results of a sample : 100 men of one battalion were examined, the number being made up of 50 men sleeping in cubicles and well spaced out small rooms ; none of these men were carriers. Thirty-four of the remainder came from one barrack-room, which was divided by a partition into inner and outer parts with 17 men in each. The inner portion was the smaller and in one place

the beds were only about 1 ft. apart. There was one carrier in the outer part and three in the inner. Therefore, although the whole sample showed the excellent carrier rate of 4 per cent., yet here was a compartment in the barracks with a carrier rate of 18 per cent., and obviously a source of potential danger.

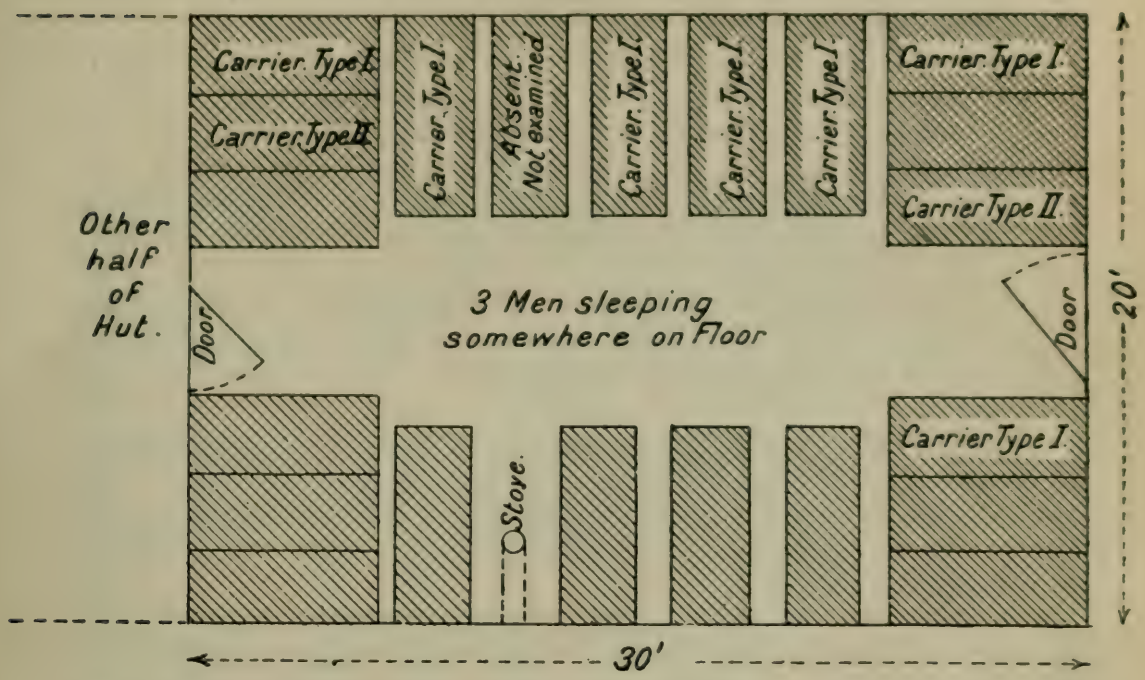


Chart IX. Cross-Infection due to Overcrowding.—Plan of half of an overcrowded hut which was divided by a complete partition (with a door) into two halves, each 30′ × 20′, showing the distribution of carriers.

In addition to the bedboards shown, all of which were occupied, three men slept on the floor.

Each man had therefore only 25 sq. ft. of floor space.

Owing to the doors, the beds on the short sides are actually in contact.

The hut was of superior type to the ordinary Army pattern, and the windows were of a different pattern.

O. *Conclusions.*

1. A high carrier rate denotes that overcrowding and dangerously unhygienic conditions prevail in the community in which it is found.

The high carrier rate wave takes from two to three weeks to develop fully after the commencement of the severe overcrowding. Catarrhal disease, by increasing the spraying capacity, is a powerful activator in the process of development. (The catarrhal symptoms are probably seldom due to the meningococcal infection itself.)

2. A wave of high (non-contact) carrier rates *precedes* and accompanies an outbreak of cerebrospinal fever.

In other words there is a carrier epidemic (for the most part entirely devoid of symptoms) *preceding* and accompanying the much smaller case epidemic.

The meningeal cases are the visible foam on the dark carrier rate wave ; when the latter reaches a certain height (20 per cent.) something happens, and cases appear, just as when a water wave reaches

a certain height the surface tension cracks, and it becomes a visible ' white horse '.

Conditions other than height of wave may modify the appearance of the foam, but a certain height is essential.

Whilst then sporadic cases may occur in a community with any carrier rate, anything approaching an epidemic of cerebrospinal fever will be heralded by a warning rise of considerable height in the carrier rate.

3. In addition to the rise in the carrier rate, the proportion of carriers of epidemic strains (i. e. those agglutinating with serums of standard type) to carriers of organisms culturally resembling the meningococcus, but not so agglutinating, will show also a very conspicuous increase previous to the commencement of the epidemic.

4. The prevalent type of meningococcus in the carrier epidemic will be the prevalent type in the case epidemic.

5. Under the same conditions of housing and overcrowding, a community, such as a large dépôt, will show substantially the same carrier rate amongst non-contacts as amongst actual contacts of cases.

6. Severe overcrowding was during the war always accompanied by a carrier rate (serologically proved) of at least 20 per cent. This percentage was adopted as the danger line in the War Office memorandum on cerebrospinal fever (March 1917), and events showed its substantial accuracy.

A carrier rate of this height invariably showed that the low standard of mobilization (40 sq. ft. per man) had been infringed, and usually it was found that the beds in the unit examined were actually less than 1 ft. apart.

A carrier rate of 20 per cent. (without awaiting the occurrence of any actual cases of the disease) should be regarded as a signal for prompt and effective action to abolish overcrowding, and to improve ventilation, and to increase the distance between the beds to at least 2½ ft.

7. Overcrowding in sleeping quarters is of most consequence, and the distance between beds is of paramount importance.

8. Carrier rates between 10 and 20 per cent. are unsatisfactory, and imply a certain amount of overcrowding ; they must be watched with suspicion.

9. Carrier rates from 2 to 5 per cent. may be considered usual under the best conditions obtainable in barracks and hutments.

10. Quite a moderate increase in the distance between beds (i. e. to 2½ ft.), combined with the simplest methods of improving ventilation (strictly enforced), proved highly efficient agents in reducing high carrier rates under ordinary circumstances, and apart from the severe pressure of war conditions 3 ft. should be regarded as the minimum distance between the edges of beds.

11. Recruits, being specially susceptible, should be specially spaced out during their first three months of service. ' Maxima debetur pueris reverentia ' is especially true in cerebrospinal fever.

The Royal Commission of 1861, who established the ' peace ' standard of accommodation, summed up the whole matter of overcrowding in the following pregnant sentence : ' If above a certain

number of men are placed in a given cubic space, the lives of some of these men and the health of others is certain to be sacrificed.'

My warmest thanks are due to my laboratory assistants, Sergeant C. S. Barrett and Corporal R. H. Piejus, to whose great technical skill and cheerful indefatigability this work owes so much.

Lt.-Colonel Sir Shirley Murphy, K.B.E., Senior Sanitary Officer, has helped me in every way in the kindest possible manner, whilst the kind help and advice of Lt.-Colonel M. H. Gordon, C.M.G., has been invaluable to me.

21. Mass-Disinfection of the Nasopharynx.

It is abundantly clear that the highway of the meningococcus is from nasopharynx to nasopharynx. Before the recent outbreak began, the work of Bruns and Hohn, and also of Netter and Debré, had shown that the carrier rate and case rate run parallel. This correspondence is confirmed by the continuous observations recorded by Captain Glover, which have also brought out a new and very important point, namely, that when the meningococcus carrier rate rises beyond a certain level—in other words, when the dosage is sufficient—and other factors, such as season and overcrowding, are favourable, cerebrospinal fever is liable to manifest itself, especially among persons newly introduced into such a community—for example, recruits. The observations of Colonel Flack and Captain Glover show in addition that the carrier rate rises before cases begin to occur, and accordingly that this index of the meningococcus ' pressure ' in a community is capable of giving valuable and timely warning of an impending outbreak. Captain Glover has shown further that the carrier rate reveals the degree to which a given community is being subjected to particulate pollution derived from the upper respiratory passages—in short, to the amount of overcrowding—and the satisfactory fall in a high carrier rate that has consistently followed his procedure of ' spacing out ' is evidence alike of the accuracy of this view, and of the short striking distance of the meningococcus.

The nasopharynx is thus both the head-quarters and the rallying point of the meningococcus, as well as apparently the primary site of infection in cerebrospinal fever, and in the genesis of outbreaks of this disease dosage appears to play an important part. But while dilution of the virus outside the body by the elimination of overcrowding is undoubtedly the best way to prevent cerebrospinal fever, this measure is not always practicable, especially under war conditions ; and since the nasopharynx is accessible for the purpose of disinfection, it was desirable to ascertain to what extent the abundance of the meningococcus in the nasopharyngeal secretion of individuals could be reduced by local disinfection, and the practical value of such procedure not merely for clearing the last meningococcus out of the nasopharynx of a chronic carrier, but more especially for holding cerebrospinal fever in check in circumstances where overcrowding is unavoidable, the carrier rate has risen to a dangerous level, and an outbreak is already threatened, or in actual progress.

Now the meningococcus is one of the least resistant of all pathogenic bacteria to disinfectants, and is rapidly destroyed by some of

the mildest of these bactericidal agents in concentrations easily tolerated by the mucous membrane of the nasopharynx. Theoretically, therefore, the meningococcus should be destroyed in the nasopharynx with ease if only it could be reached there by a suitable disinfectant. Apart also from its actual destruction or impairment by disinfectants, the experimental observations described previously on the pathogenicity of the meningococcus prove that the vitality, reducing power, and virulence of this micro-organism alike undergo a rapid decline when the medium in which it is suspended is unfavourable.

At an early stage of the epidemic in response to demands for a local disinfectant for use prophylactically in the army during outbreaks of cerebrospinal fever, it was recommended that a warm solution consisting of one part potassium permanganate in 5,000 parts of normal saline should be sniffed up through one nostril at a time and expelled through the mouth, the exercise being completed by gargling some of this solution. When carrying out the insufflation it was often found convenient to adopt a procedure recommended by Colonel Flack, and for each man to sniff up the solution from the palm of his hand into which it had been poured previously by the N.C.O. in charge. The selection of permanganate was based on the result of some comparative trials of the value of a series of disinfectants when gargled for actually reducing the number of the bacteria normally present in saliva ; and this disinfectant had the further advantage that it is known to be destructive to filtrable virus ; so that the treatment was also available for prophylactic use during outbreaks of measles. This method of personal prophylaxis was subsequently adopted by the Local Government Board, being recommended by them for a similar purpose during the recent epidemic of influenza. The value of the treatment depends on the thoroughness with which it is carried out, and the results reported so far have been encouraging.

It is conceivable that the local application of a disinfectant may reduce the abundance of the meningococcus in the nasopharyngeal secretion in one or more of the following ways, viz.: (1) by washing the infected secretion off the mucous membrane ; (2) by stimulating an increased flow of mucus ; (3) by rendering the secretion on the mucous membrane bactericidal to the meningococcus, and (4) by producing a certain amount of local irritation, thus promoting a freer circulation of lymph and accelerating the natural process whereby the mucosa frees itself of the meningococcus. It would seem possible, and even likely, that several of these effects may be brought about at the same time, and that the local application of a disinfectant may produce changes over and above those due to its bactericidal action alone.

A disinfectant in concentration sufficient to destroy the meningococcus without injury to the mucous membrane may be conveyed to the nasopharynx from without in one or more of the following ways :

(1) In bulk, as in the douche, &c.

(2) In droplet form, as by the ordinary hand-spray or atomizer.

(3) In the form of fine droplets floating as a cloud, mist, or nebula, mixed with steam derived from an automatic spray actuated by steam under pressure.

(4) In the form of vapour or of gas.

As procedures (1) and (2) had already received considerable trial, and the chief requirement was for a method whereby a suitable disinfectant could be brought in contact with the nasopharynx of as large a number of men at a time as possible, an attempt was made to elaborate a practical procedure of applying disinfectant in the manner described under (3). The claim of Kuster that meningococcus carriers had been successfully treated by a similar method elsewhere (*Deutsche med. Wchnschr.*, Sept. 9, 1915), was an additional incentive to the present inquiry.

A. *Preliminary Experiments.*

The problem for investigation was formulated as follows :

(1) Does the air of a room when densely charged with a solution of a suitable disinfectant in a finely divided state exert bactericidal properties ?

(2) If so, to what extent can such air be tolerated by human beings inhaling it ?

(3) Is the method a practical one for destroying the meningococcus in the nasopharynx of carriers ?

The first point to be examined was the question whether the air of a room becomes bactericidal when charged with a solution of disinfectant in a finely divided state. The experiments were made in a room kindly put at our disposal for the purpose by Major-General Sir David Bruce, K.C.B., F.R.S., at that time Commandant at the R.A.M. College. The capacity of this room was approximately 1,000 cubic feet. Permanganate being unsuitable because of its staining effect, the disinfectants chosen for trial were chloramine T. (Dakin) in a concentration of 1 : 50 or 1 : 100, and trial was also made of zinc sulphate 1 : 100. Both chloramine and zinc sulphate were found to rapidly destroy the meningococcus *in vitro* at these concentrations.

For the purpose of keeping the air of the room charged with disinfectant, a specimen of the Lingner sprayer was used which was kindly lent to us by Dr. A. E. Thomas, Medical Officer of Health of Finsbury. The Lingner sprayer, originally designed for disinfecting a room with formalin vapour, consists of two essential parts. The first of these is a ring-shaped copper boiler, in form not unlike a bicycle tyre, fixed immediately over a circular trough which is filled with asbestos wool moistened with spirit. From the boiler three pipes convey steam centrally to a chamber of about two litres capacity, in which the solution of disinfectant is placed. The roof of this chamber carries a safety valve, and four spray nozzles which point vertically upwards. In practice the boiler of the spray is first filled with 1,500 c.cm of water, and then the disinfectant chamber is charged with 1,500 c.cm. of solution to be tested. Owing to the powerful nature of the burner, and to the efficiency of the boiler, the sprays begin to work within about ten minutes of lighting the burner. The dry bulb temperature of the room rapidly rises, followed by that of the wet bulb. In about ten minutes after the sprays have started (that is, twenty minutes after lighting up), the temperature of the two thermometers becomes equal, indicating that the air is saturated.

Owing to the comparatively small size of the instrument the sprays in the Lingner apparatus cease work after running for about forty minutes. Thus the air of the room could not, with this apparatus, be kept saturated with the disinfectant vapour for over twenty minutes.

For determining the bactericidal capacity of the air when charged with disinfectant, *Staphylococcus epidermidis* was used both from its convenience, and also because it is hardier and more resistant to disinfectants than the meningococcus. Equal amounts of a broth culture of the staphylococcus were spread over a number of agar plates. One of these plates was kept as a control, while the others were exposed for varying times to the air of the room. The amount of staphylococcus destroyed was readily seen by incubating the plates for 24 hours at 37° C., and then comparing the amount of growth of the staphylococcus on the plates that had been exposed to the air with that on the control plate.

It was found that when the sprayer was in good working order, and the air of the room so densely charged with steam and droplets of disinfectant from the sprayer that the hand could not be seen in front of the face, and 2 per cent. of chloramine was being sprayed, the staphylococcus was killed in practically all the plates within 5–10 minutes.

The bactericidal action of air charged with 1 per cent. of chloramine was also well marked, and though less powerful than the chloramine, air charged with 1 per cent. of zinc sulphate was found to possess considerable bactericidal powers also. The bactericidal action of the air began with the spray, was most intense during the second five minutes, and ceased within two minutes of the sprays being stopped. It was observed further that the disinfecting property was apparently confined to the actual droplets of disinfectant floating in the air ; since inverted plates sown with the staphylococcus were unaffected.

The next step was to ascertain the extent to which the air of the room could be tolerated when the sprays were in full blast. This was investigated by sitting in the room and inhaling the air vigorously through the nose while the sprays were in action. When 2 per cent. of chloramine was being sprayed it was found that the atmosphere could easily be tolerated for 5–10 minutes, and that some persons could stand it for as long as 40 minutes without discomfort. The chloramine, however, was found to be more trying than zinc sulphate, and as in course of these observations repeated and prolonged exposure to 2 per cent. chloramine was found to produce in some persons headache and rise of temperature, and such effects were never observed with 1 per cent. zinc sulphate, the latter was chiefly used later on when applying the method to large bodies of men.

The value of the method was next investigated for reducing the abundance of the meningococcus in the nasopharyngeal secretion of carriers. The nasopharynx of two known carriers of the meningococcus was swabbed, and they were then invited to inhale vigorously through the nose the spray-laden air of the inhaling-room for a period of five minutes and upwards, after which they were reswabbed. Both of these carriers were most anxious to get rid of the meningococcus,

and co-operated to the utmost of their power in the treatment. It was found that whereas before entering the inhaling room the nasopharyngeal secretion of both alike gave a profuse growth of the meningococcus, after the sojourn in the inhaling-room their nasopharyngeal secretion in both cases showed no meningococcus colonies at all ; the plates now only giving a few colonies of streptococcus or pneumococcus. Inhalation of the spray-laden air of the inhaling-room therefore was capable of producing temporarily a very material reduction in the abundance of the meningococcus in the nasopharyngeal secretion of carriers.

B. *Observations on the Value of Inhaling-Room Treatment for clearing up Meningococcus Carriers.*

(*a*) *Results with Chloramine T.*

Having obtained in this way a method of disinfecting the nasopharynx that was capable of being applied on a large scale, the treatment was tried first of all on 13 chronic carriers in the London District. By means of canvas screens the size of the room was reduced to an area of 750 cubic ft., and in this space one litre of 2 per cent. chloramine was sprayed in the course of 15–20 minutes, during which time the carriers remained in the room close to the sprayer, vigorously inhaling the air through the nostrils. This treatment was carried out once a day, and each man was swabbed before entering and after leaving the inhaling-room. The results were most encouraging, since although all of the 13 carriers were heavily infected at the start, and seven of them had been carrying for from 6–17 weeks, no less than ten of them became free of the meningococcus in from 6–10 days after beginning the treatment. In each case six successive negative swabs were obtained before discharge, and five of these men were re-examined 5–7 days after discharge and found to be still negative. On the other hand, three of these chronic carriers failed to clear up ; one of them was super-sensitive to the chloramine and could not go on with the treatment ; the other two could not be induced to inhale properly.

It was noticed during these and other observations made at this time that the men who were ardently desirous of becoming free of the meningococcus cleared up as a rule more quickly than the others—a result probably due to the zeal with which they co-operated in the treatment, and the vigour with which they inhaled the spray-laden air through the nostrils while they were in the inhaling-room.

(*b*) *Results with Zinc Sulphate.*

The solution of zinc sulphate tried was 1·2 per cent. This spray was less trying than the 2 per cent. solution of chloramine, but the results were not so good. Ten carriers whose nasopharynx was only lightly infected with the meningococcus cleared up after from 2–8 inhalations, but ten others who were more heavily infected at the start did not clear up until they had received from 8–42 inhalations. The results therefore with zinc sulphate were inferior to those obtained with chloramine.

These observations on the value of the inhaling-room treatment for clearing up chronic carriers, especially those with chloramine, were distinctly encouraging, and it was hoped that the method would prove of general service for this purpose. This hope, however, has not been fulfilled. The method has been tried on carriers by Glover in the London District, by Embleton at Netley, and by Fildes at Haslar, who made extremely careful controls, all observers alike with but indifferent results. A possible explanation of this lack of success is that, as was found by Cleminson, a large proportion of chronic meningococcus carriers suffer from adenoids or from a condition of ' firm mucous contact ', leading to obstruction of the nasal passages ; conditions which tend to prevent full access of the disinfectant through the nose to the infected surfaces. Another is that the carriers tested by Flack and myself were subjected to the treatment during April, May, and June, when the general carrier rate usually tends to fall ; but the observations with zinc sulphate served as a control on this point. A third explanation is that our first carriers were a set of guardsmen most of whom were particularly anxious to become free of the meningococcus and co-operated most heartily in the treatment. The importance of this point cannot be overstated, since unless every care is given to keep the atmosphere fully charged with the spray, and the men inhale as vigorously as possible through the nose, the disinfectant does not reach the mucosa of the nasopharynx in sufficient quantity to produce the best results.

On looking back at our early experiments on carriers also, a point comes to notice that escaped attention at the time. These carriers were all living in isolation together, and those who became free under the treatment continued to associate with the others until found negative on six successive examinations before and after leaving the inhaling-room. Now if these men had been freed of the meningococcus solely by the disinfectant, how is it that they did not become reinfected by the other carriers ? On the whole, the most likely explanation would appear to be that the inhaling-room treatment—especially with chloramine—acted not only as a disinfecting process, but that by increasing the local circulation of lymph, it also accelerated the natural process whereby carriers in course of time become free of the meningococcus.

C. *The Value of Inhaling-Room Treatment for Mass-Disinfection of the Nasopharynx.*

While the early promise of the inhaling-room treatment for the purpose of freeing chronic carriers of the meningococcus has not been fulfilled, there can be no doubt as to the capacity of this treatment to effect a temporary reduction in the number of meningococci in nasopharyngeal secretion. Now according to the time that they take to clear up, carriers of the meningococcus are distinguishable into two classes as temporary or chronic ; and from observations made in course of actual outbreaks of cerebrospinal fever among troops, it was found that during the period of the year when carriers and cases alike were at their highest level, the majority of the carriers were of the temporary class. From the point of view of mass-infection there-

fore the temporary carrier is of chief importance : a minority consisting of chronic carriers being mainly responsible for conserving the infection, and for carrying it over from one season to another.

Accordingly, for the purpose of dealing with actual outbreaks of cerebrospinal fever among troops undergoing intensive training in circumstances where overcrowding is unavoidable and the carrier rate has risen to a high level, a method of nasopharyngeal disinfection was required that could be applied to a large number of men at a time without interfering with their military training. The inhaling-room method appeared suitable for trial in such cases, and as the zinc sulphate spray was better tolerated than chloramine, and temporary carriers had rapidly cleared up under it, the zinc salt was chosen for this purpose, chloramine being reserved for special cases. A further advantage of the zinc salt was that the inhalation of atomized zinc sulphate solution mixed with steam had been found to have a beneficial effect on carriers suffering from catarrh, who in general are far more active in disseminating the meningococcus than other carriers.

(i) The first report of the result of practical trial of the steam atomizer charged with 1 per cent. solution of zinc sulphate for the purpose of checking the spread of cerebrospinal fever was published by the D. D. M. S. of the New Zealand Expeditionary Force in the *British Medical Journal* of February 24, 1917. The memorandum in question stated that in conjunction with the segregation of carriers, this method of nasopharyngeal disinfection had proved successful in checking the disease which had previously caused much trouble both in training camps in New Zealand, and on board transports conveying troops from that Dominion.

(ii) A severe test of the zinc sulphate spray was made in case of an outbreak of cerebrospinal fever in a large training dépôt during the month of February, 1917. At the time when the treatment was applied 5,200 men were under training at this dépôt, and examination of contacts of the cases and also of samples swabbed from the rest of the dépôt indicated a mean carrier rate there of approximately 60 per cent. As over half of the men were presumably carriers, and the disease in the case of those who had developed meningitis was of a virulent type, an attempt was made to disinfect the nasopharynx of the whole garrison. Four inhaling-rooms were improvised by partitioning off the central portions of four empty huts ; the part of the hut on either side of the inhaling-room being used as waiting-room and cooling-room respectively. Two Levick steam atomizers were installed in each inhaling-room, and 1 per cent. zinc sulphate dissolved in 0·85 per cent. saline was sprayed continuously. The men were marched through these huts in the same manner as for steam baths at the front, and each man remained in the inhaling-room for five minutes, during which time he was instructed to inhale the atmosphere vigorously through his nose. After undergoing this treatment once daily for a week, an interval of three days was allowed to elapse without spraying, and then the garrison was sampled by swabbing 50 men from each of the five battalions composing it. The carrier rate was now found to be 30 per cent. As this was still too high, the garrison was subjected to further spraying for a week and then sampled again. The carrier rate was now 8 per cent. ; but this

was probably too low a figure as the weather was very cold and some of the plates were unavoidably chilled during their conveyance to the laboratory. A sample taken a fortnight later showed a carrier rate of 20 per cent.

It may be of interest to mention that immediately before the spraying, 100 per cent. of the sample of non-contacts examined showed Gram-negative cocci in their nasopharyngeal secretion, whereas in the sample of the whole dépôt taken after the second week's spraying only 10 per cent. of the men showed colonies of Gram-negative cocci.

It was observed that the treatment of the whole population by the steam zinc sulphate solution spray (for two periods of seven days, with an interval of a fortnight) was followed by a temporary cessation of cases on each occasion. Further particulars of this outbreak have been described by Captain Glover in the R.A.M.C. *Journal* of January 1918.

(iii) In a camp of the R.F.C. near London a fulminating case of cerebrospinal fever occurred and 48 contacts with it gave a carrier rate of 10·4 per cent. Four days later a sample of 64 men (non-contacts) from this camp showed a carrier rate of 31·2 per cent. A spray was now installed, a very efficient R.F.C. sergeant took charge of it, and all of the men in the camp were put through the inhaling-room. Two per cent. of chloramine was sprayed, and each man remained in the room for 15 minutes, during which time he was urged by the sergeant to inhale vigorously through the nose. In order to test the efficiency of the disinfection, Major Tulloch, under whose supervision the treatment was carried out, made a surprise visit after the spray had been going for three days, and swabbed 41 men who had recently been through the inhaling-room. Not a single one of them was positive. The spraying was continued for a fortnight, and then after an interval of three days 73 men were swabbed. The carrier rate was 21·9 per cent. No further cases occurred after the spray was installed in this camp.

It appeared from these practical trials, and also from others over the same period when the inhaling-room treatment was put into operation during an outbreak of cerebrospinal fever—but the bacteriological observations were less complete—that the application of this procedure was followed by an apparent drop in the case incidence and by a fall in the carrier rate, and the treatment had the great merit that it did not interfere with the military training of the men. The fall in the carrier rate, however, was strictly limited. After the spraying was stopped a considerable residue of carriers remained upon whom the disinfection was ineffective, or at the most only temporarily effective. Nevertheless, because of its value for dealing with mass-infection during an outbreak it was clear that the inhaling-room method had more than justified itself, and was worthy of trial on a larger scale.

From an early stage of these experiments efforts were made to obtain a sprayer that would be more efficient than the Lingner apparatus, and free from the heat-producing and other disadvantages of that instrument. An adjustable apparatus operated by steam under high pressure and designed by Lieut. E. Gordon, R.E., was tried for some time and served to bring out the important point

that for disinfection of the nasopharynx it is advisable to use a somewhat coarse spray, because if the division of the disinfecting fluid is too fine it penetrates to the bronchi and, especially in the case of chloramine, is apt to excite considerable coughing. When through illness Lieut. Gordon was unable to continue the work Major Hine took up the subject, and after many experiments elaborated a special jet for use with high-pressure steam generated in a boiler outside the inhaling-room. Having elaborated a suitable jet Major Hine then gave his attention to designing an inhaling-room that would permit of the disinfection of a larger number of men at a time than was possible with the procedure previously in use. The developments resulting from these improvements are described by Major Hine.

22. Improved Atomizer for Mass-Disinfection.

By T. G. M. Hine, O.B.E., M.D., Major R.A.M.C.

As Lt.-Col. Gordon has mentioned above, the Lingner Sprayer—or as it was called later, when improved by us, the Levick—had certain disadvantages in regard to the amount of heat set free and the comparatively small area over which the spray-mist was distributed. This led me to contrive a spray-jet which we hoped would be capable of dealing efficiently with much larger numbers of men and giving a much cooler atmosphere.

It may, perhaps, be as well to describe fairly fully the construction of the jet first of all.

I commenced by having several patterns made of other types of steam-spraying devices, worked by a small model boiler made for me by Messrs. Bassett-Lowke of Northampton and London. All of these were more or less successful, but the ' scent-spray ' pattern of arrangement of jets gave the greatest immediate promise of success. The small boiler I used raised steam by a couple of Primus burners with a controllable range of from 10 to 80 lb. A pressure of 40 to 50 lb. can generally be obtained wherever a steam installation is already at work, and, should a boiler have to be specially procured, one working at this low pressure as present practice goes, would be initially cheaper and more easily maintained.

The object of the experiments was to devise a jet, worked by steam and giving a controllable fineness of disinfectant droplets in a regulated amount, which could be installed in numbers in a room to fill the air completely and quickly with a mist of disinfectant solution, and to keep this up for any desired time. The boiler being outside, the room would not be expected to get too warm, and the steam would also be immediately cooled and condensed by contact with the cold disinfectant.

This ' scent-spray ' type of jet was that used in Lister's original carbolic spray. When applied, however, to my purpose it was found to give irregular results. There was no certainty, for instance, when starting, that the steam would not blow down the suction jet (set at a right-angle) into the disinfectant instead of sucking it up ; this often happened. The projection of the spray to a distance was rather feeble, even though a considerable pressure were used, while the regulation of the amount of fluid discharged was only nominal.

Several different patterns of nozzles were tried, from an obtuse cone to one of extreme acuity. The sizes of the openings proved of much importance, and many variations and combinations of these were tested. A jet also was made in which the angle between the nozzles could be varied. Ultimately a combination was arrived at which would project a fairly fine and beautifully divided mist a distance of at least 15 ft. with a pressure of 45 lb., while at 55 lb. it reached quite 20 ft. The spray is in the shape of a trumpet-shaped cone, rapidly enlarging, so that at 15 ft. the mist can be perceived over an area of 10 ft. wide or more. In this condition it remains suspended in the air an appreciable time. If, however, the spray be intercepted between the nozzle and a distance of up to 6 ft. away, i. e. in its first concentrated portion, it rapidly collects and falls off the obstacle in a shower.

The dimensions of these jet nozzles are, for the steam one, ·047 in. at the exit and for the suction jet ·037 in. This latter is a simple cone brought to a feather edge at the point, while the steam cone, in the latest pattern, is more complicated, having an internal cone, a short cylindrical portion near the orifice, and, finally, for certain purposes, a slight bell-mouth. More metal at the exit is allowable in this nozzle and is provided for strength.

The angle between these nozzles has been determined to be the best when made of 84°, a steady irreversible flow being thus ensured.

The adjustment is very simple. The suction tube is tapped exteriorly with a fine thread and is furnished with a hexagonal nut. This threaded portion passes through a slightly smaller cylindrical threaded and slotted passage in the supporting bracket, and by turning the tube, by a spanner applied to the nut, the suction jet can be raised or lowered and brought into any desired degree of apposition with the fixed steam nozzle. The slot in this passage allows the outer and smaller passage in the metal bracket to open and contract round the screwed suction tube, holding it firmly and automatically, preventing play and back-lash or the unintentional alteration of the adjustment. The whole of this portion has to be very accurately made, so that the axes of the nozzles lie immovably in the same plane.

Both the nozzles, and especially the suction one, are liable to damage if bruised or to become blocked. Simple provision has been made for their removal and, if necessary, replacement.

With regard to the physical conditions governing the action of the jet, by analogy with the scent-spray it would at first appear that the current of steam created an area of diminished pressure at the top of the suction nozzle by its mere velocity, thus sucking up and dispersing the fluid. In proof that this is not the whole story, however, I quote the following instructive incident. As will be mentioned later, the naval authorities have been interested in the possibilities of this method and have installed it in most of their dépôts and many ships. A difficulty in the latter case arose as to the employment of steam of the necessary pressure, for certain technical reasons. I thought of compressed air, of which there is always a good supply for torpedo work. It was determined to try the jet under these conditions, and by arrangement with the Admiralty

and the courtesy of Commodore H. d'E. Skipwith, R.N., of H.M.S. *Vernon*, commanding the torpedo and mining school at Portsmouth, I was enabled to try some experiments with compressed air. I first fixed up a new jet not yet tested, but could not eject more than 20 c.cm. per minute even with a pressure of 70 lb. of air. I then substituted an older pattern well tested, with the same want of success. It was obvious at once that the conditions differed, and that the steam condensing in contact with the cold fluid apparently caused a greater diminution of pressure at the jet point than that caused by its mere velocity. A suitable enlargement of the suction jet, allowing more fluid to pass, remedied the deficiency and an equally good result as with steam was obtained.

After this preliminary description of the inception of the spray-jet and its action, I will content myself by stating that I made a number of experiments showing the form of the spray emitted by the jet and its disinfectant effect on plates of staphylococcus, in which all the plates in the radius of action of the spray were sterilized, nearly completely, in ten minutes with a $1\frac{1}{2}$ per cent. solution of zinc sulphate. These experiments are embodied in a report to the Medical Research Committee which I submitted in August 1918.

It then remained to test its practical application on a sufficiently large scale. For this purpose the Committee offered to issue it free of cost in numbers sufficient to give it a fair trial, and I therefore decided to send it out to various points as widely distributed and as numerous as possible, so that the conditions might differ and that there might be a good chance of an installation being situated in at least one or two places where an epidemic might reasonably be expected to break out.

I divided 300 of these jets among the Army, Navy, Air Force, and a few civilians, while the colonial troops also had a share. Several spraying chambers were fitted up at various points, some of which I myself helped to instal, and Army huts of the usual type, i. e. 60 ft. × 15 ft., were commonly used, the Naval rooms being, however, often specially built and on a larger scale. These rooms had a small boiler outside for steam, and from ten to twenty jets, according to the size of the room, were fitted inside, and arranged so that the sprays were directed alternately from each side ; in a very short time, about one minute, after these jets were started, the hut was filled with a dense disinfectant mist. In these spraying chambers, when carefully managed, it was found possible to disinfect between 400 and 500 men per hour, allowing every man ten minutes to inhale.

Although I have not received, as yet, more than a few preliminary reports on the action of this spray-jet, in those that are to hand the reporters generally seem to be of the opinion that epidemics have to some extent either been checked or prevented, although it has naturally been difficult for them to be certain on the point owing to various indeterminate factors. They are all clear, however, that this form of spraying apparatus is easy to control and thoroughly satisfactory as a spray-producer. The chief doubt seems to be the exact value of spraying disinfectants at all. As nothing but far wider experience can determine fully this point, about which also Col. Gordon has an open mind, there is no need for me to discuss it further.

The Admiralty, it is true, appeared to be convinced of the value of this form of treatment, especially in very crowded places such as transports (which the experience of the New Zealand authorities would seem to support), for they issued an order that all ships of this class leaving this country should be fitted with a spraying room using this system. Altogether over 200 transports and about 40 ships of the Royal Navy were fitted with spraying rooms before I had to stop personally testing and distributing these jets on being demobilized. More than 1,000 jets were asked for, made, and issued through me, beyond the 300 which were originally provided by the Medical Research Committee. The American Authorities also procured sufficient jets for about eight spraying rooms.

The above is only a very bald description of the work that was done in connexion with the jets, but I cannot refrain, in conclusion, from mentioning the great assistance the sole makers, Messrs. Bassett-Lowke of Northampton, were to me throughout, during both the experimental and manufacturing stages, and especially the fact, that, by arrangement with me, they supplied the armed forces of the Crown with these jets at cost price.

23. An Analysis of the result of the Therapeutic Application of Monotypical Antimeningococcus Serum in 267 cases of Cerebrospinal Fever.

By T. G. M. Hine, O.B.E., M.D., Major R.A.M.C.

In February 1919 a White Paper was issued by the Medical Research Committee containing an analysis by me of the first 90 cases of cerebrospinal fever treated with monotypical serum. The serum in question had been prepared on behalf of the Medical Research Committee and the War Office by Dr. Stanley Griffith at Cambridge, acting in co-operation with the staff of the Central C.S.F. Laboratory in London.

Since the publication referred to, reports on 177 further cases have been received, and in the present paper I propose to combine these with the reports on the first 90 cases, thus analysing 267 cases in all. I wish at once to express my thanks to the forty reporters for the care they have taken to procure the details requested.

The White Paper began with an introduction by Lieut.-Col. Gordon, in which he described the train of circumstances that led to the preparation of monotypical antimeningococcus serum for therapeutic trial in cases of cerebrospinal fever. For the benefit of those who have not had the opportunity of reading this White Paper, it may perhaps be as well to summarize his remarks, adding the conclusions drawn by myself after analysing the clinical results then available.

The mortality from cerebrospinal fever with the ' multivalent ' serum previously in use was still so high that an attempt to improve the efficacy of the serum was very desirable ; and the fact that a particular consignment of the multivalent serum had been found to give far better results clinically than the remainder indicated that success was within reach. The Medical Research Committee therefore deputed Dr. Stanley Griffith in their employ at Cambridge (where it was

possible to keep the necessary horses) to co-operate with us in an attempt to prepare serum of the potency desired.

Experience during preceding stages of the outbreak and serological investigation of meningococci isolated from the cases led us to start at the beginning, and to prepare individual horses against meningococci belonging to a single serological type rather than to inject the animal with representatives of more than one type: hence the monotypical serum. As 80 per cent. of the cases were known to be due to meningococci belonging to one or other of the first two groups, a pooled serum consisting of equal parts of the antisera to Types I and II respectively was put up for use as a routine treatment before the type of the infecting meningococcus could be determined. After referring to the procedure followed by Dr. Griffith in preparing the horses, Colonel Gordon proceeded to describe a laboratory standard of potency which investigations, carried out in the Central C.S.F. Laboratory during preceding stages of the outbreak, indicated that the serum should satisfy before being issued. The principal test was briefly that 0·5 c.c. of the serum should show experimental evidence of neutralizing the endotoxin of a meningococcus representative of the type against which the serum had been prepared. Having passed the laboratory tests, the serum was issued in the first place to a number of selected observers, who had special facilities for trying it clinically, with the request that they would report on the results.

The results shown by the first 90 cases, when analysed, were very encouraging, especially with regard to the cases infected by Type I meningococcus, previously one of the most fatal forms of the disease, for out of 34 Type I cases submitted to treatment with the monotypical serum no less than 33 had recovered. The results with the Type III serum were also excellent; out of ten cases infected by meningococci of this type all had recovered when treated with the homologous serum; it was significant, however, that several of these Type III cases had failed to benefit from the pooled serum consisting of equal parts of Type I and II antisera with which they were first treated, and only began to improve when given the Type III serum. On the other hand, the results with the Type II serum were less satisfactory, for out of 32 Type II cases seven had died. In commenting on these figures, Colonel Gordon pointed out that the success of the Type I serum was in accordance with the serological uniformity of meningococci belonging to this type, and the less satisfactory result with the Type II serum was in correspondence with the more complex character of the group serologically and with the circumstance that, in spite of every effort, the serum prepared against Type II had been found in laboratory tests to be weaker relatively in anti-endotoxin than the serum prepared against Type I. Although the number of cases dealt with was small, the fact that a considerable proportion of them were severe—some very severe—added weight to the encouraging evidence afforded by them as to the therapeutic value of the serum.

Particulars of the analysis of the 90 cases were set out in a series of tables compiled by me from the reports, brief details being given of each case under review. From a study of the information thus afforded I arrived at three conclusions, as follows: (1) The importance

of giving the specific serum as early as possible. (2) The necessity of repeating the dose every day until all danger of relapse had passed. (3) The great importance of determining promptly the type of coccus concerned, and of using at once the appropriate serum. Finally I suggested that since the Type II serum did not contain so much antiendotoxin as the others, it was advisable to push the dose of this both in quantity and frequency.

After this brief recapitulation of the salient points of this White Paper we can now commence the study of the complete series of 267 cases of Cerebrospinal Fever.

The experience gained from the study of the first 90 cases, together with the impressions I received during a preliminary inspection of the new ones, but before any detailed examination had been commenced, led me to believe that the classification previously adopted might be modified with advantage, and that a fresh classification would give a more correct representation of the actual facts. My reason for thinking this was that the preliminary rough inspection showed a larger proportion of cases in which the serum was administered ' in the dark ', either because the type had not been determined at all, or so late as to be of no practical value in treatment. This occurred because either facilities for typing did not exist, or the coccus could not be grown, or the report from the laboratory to the clinician was unduly delayed. Thus several cases treated with pooled I and II serum alone proved eventually to be Type III cases, or, if a Type I or II case continued to be treated throughout with the pooled serum alone, it received not more than half of the appropriate antiendotoxin in each dose. Again, there was a certain number in which the wrong type of serum was proved to have been used. It appeared to me, therefore, more reasonable to take these cases out of the first two tables in the previous report and place them in a separate table—a class by themselves.

I have again partially adopted the system, which Dopter used, of first separating those cases which died, having another severe intercurrent disease sufficient to cause death by itself and not obviously connected with C.S.F. I have not, however, gone to his length of also removing moribund cases, which I do not regard as an admissible procedure in this kind of classification (although the point may be arguable), for some of the so-called moribund cases have recovered under good treatment ; hence my objection. Undoubtedly, however, some of these cases were so sudden and severe as to appear doomed from the first. Although these moribund cases are included in the first table I have put them under a special heading.

The new classification is then as follows :

Table I (the old Tables I and II combined), containing in Group A the cases in which serum has been given before the 7th day, and in Group B the cases in which serum was given on the 7th day or later, or in which serum was administered with marked insufficiency, although perhaps it may have been given before the 7th day. Group B also includes the moribund cases. In all these cases the type of organism had been ascertained within a reasonable time.

Table II contains those cases in which either the type of coccus was never determined, or was determined too late to be of value,

e.g. after death, or in which the wrong serum was given. (This table does not appear in the first analysis.)

Table III (as before) contains cases dying with other serious diseases.

The above three tables contain every case reported which was treated with the M.R.C. serum, either alone or in combination with other sera—even cases only receiving one dose of this serum have been included.

In the White Paper these three tables were printed in full, but the number of additional cases has made them so unwieldy and the information to be obtained from them is so scanty, perforce, as compared with the detailed information afforded by the original reports, that Tables I and II are not here printed *in extenso*, and I content myself, therefore, by only giving a summary. Table III, which contains those cases dying with other serious diseases, has been printed in full in order that readers may see for themselves the grounds on which I justify their exclusion from Table I.

There are six more tables, all of which have been brought up to date. Tables IV, V, and VI are the same as in the first analysis ; but Tables IV and V are not shown for reasons explained later. Table VI, however, is given in full, as it is of great importance, containing, as it does, the ' Type Case Rate ' and Mortality figures.

Table VII is a similar table to VI and abstracted from it, showing the type case rate among children under 15 years of age.

Table VIII is a new table, showing the mortality of the cases in Table I (groups A and B) according to the day of the disease on which they received their first dose of serum.

Table IX, also new, compares the differing mortalities as reported by some of the medical officers in charge of cases.

Having described my new scheme of classification, we can now examine the matter contained in the tables more in detail.

From the complete series of 267 cases of the disease now collected, we must subtract the 18 cases in Table III dying from or with another serious intercurrent disease, sufficient to cause death by itself ; we are thus left with a total of 249 cases. These 249 show a mortality of 31·72 per cent. (79 deaths). This rate does not compare very favourably with the 12 per cent. mortality in the previous series. Among these 249 cases, however, are included the 48 ' untyped ' cases, or cases not receiving the right serum, having 22 deaths ; these have been placed in Table II. As previously pointed out, it does not appear fair to this particular method of treatment, based on the principle of giving a serum definitely made to neutralize the endotoxin of one type only, to include these cases ; they were, in fact, not thus treated. It appears necessary, therefore, to exclude these cases ; in the first 90 cases there were only 7 ' untyped ', but in the present series there are 48, or more than six times as many. We are, therefore, now left with 201 cases, treated by the method we are here concerned with, having a mortality of 28·36 per cent. (57 deaths).

As will be seen on referring to the Type Mortality Table (VI), however, we have 65 Type I cases with a mortality of 9·23 per cent. and 104 Type II cases, mortality nearly 40 per cent., as against 34 and 32 respectively in the first 90 cases ; in other words, there is a large

increase in the particular type of case which has all along shown the heaviest mortality, and which would largely account for the increase in the general death rate. Be this as it may, there is a big increase in the death rate over that in the first series.

A fresh factor was, however, now in operation which did not obtain in the first series and which has almost certainly had some influence on the higher death rate, namely an epidemic of influenza. This disease was not prevalent when the first 90 cases were reported, or, at least, little mention is made of it by the very careful medical officers who then sent in their results. A very large proportion of the new cases is described as suffering from influenza, or as just recovering from it. There can be no question that this must be a very serious handicap to a patient suffering from cerebrospinal fever; and though it is not my business here to discuss the influence of influenza as a precursor of this disease, it cannot fail to have an effect on the death rate, when it is remembered what an abnormally high death rate influenza has recently had of its own.

Table I, Group A, contains 141 cases treated with serum before the seventh day; 103 of these cases were treated with M.R.C. serum alone, and have a mortality of 19·41 per cent. (20 deaths), and 38 received M.R.C. serum in combination with another serum, the mortality being 18·42 per cent. (7 deaths): the total for the whole being a 19·14 per cent. mortality. Group B (the same as the old Table II) shows 39 cases in which the administration of serum had been delayed, with 38·46 per cent. mortality (15 deaths), i.e. on the seventh day of the disease or later; ten cases, 90 per cent. mortality, receiving markedly insufficient dosage, and eleven cases, mortality 54·5 per cent. (6 deaths), which were reported to be moribund on admission or having some serious complication, though probably not sufficient to cause death by itself (influenza, however, being so frequent, has not been accounted as a 'serious complication' in this series, though, no doubt, it often was).

The total mortality for these three subdivisions of Group B is exactly 50 per cent. The difference between these two groups, A and B, is striking, viz. 19 and 50 per cent., and seems fully to warrant the conclusions drawn by me in the first paper as to the dangers of delayed, erratic, or insufficient administration of serum.

It is perhaps worth mentioning, at this juncture, that not a few cases placed in Group A have also suffered, though to a less extent, from one or more of these three dangers. The extent was insufficient in my judgment to warrant placing them in Group B, but it has occurred to me that if all the cases in this Group A had received the same assiduity of treatment that was accorded to many of them, the death rate might have been still further reduced.

Table II contains those cases which were untyped or in which the type was determined too late. They are 48 in number with a mortality of 45·83 per cent. (22 deaths). A very large proportion of the severe cases in this category died (20 out of 31), but there are also naturally some mild cases which might be expected to recover in any event, and of these only two out of seventeen died. All these cases had doses of pooled I and II serum, and possibly the limited dose of antiendotoxin contained therein was sufficient to turn the tide in the

favour of the mild case, but not enough to avert the fatal result in the more serious one.

I now show a synopsis of the figures referred to above, in tabular form, with Table III *in extenso*, as already explained.

SYNOPSIS OF TABLES I, II, AND III.

TABLE I.	*Cases.*	*Deaths.*		*Mortality percentage.*		
Group A:						
Cases in which serum was first given on the 6th day or earlier. — M.R.C. serum alone.	103	20	=	19·41	19·14	
Cases in which serum was first given on the 6th day or earlier. — M.R.C. serum with another serum	38	7	=	18·42		
Group B:						23·65
Delayed exhibition	39	15	=	38·46	50·00	
Insufficient dosage	10	9	=	90		
Moribund or with severe complications	11	6	=	54·54		
TABLE II.						
Type of coccus not determined, or too late	48	22	=	45·83		
	249	79	=	31·72		
TABLE III.						
Cases dying of, or with, another severe disease.	18	18				
Total	267	97	=	36·32		

TABLE III (IN FULL).

No.	*Type.*		*Reporter's notes on case and cause of death.*
2	I	V.S.	Trench nephritis and shell-shock. Sev. gastric crises. Not expected to recover from the first.
11	II	S.	Recovered C.S.F. Died severe bronchitis later. Child.
18	II	Mild.	Died later with influenzal broncho-pneumonia.
24	III	V.S.	Died with old endocarditis, pulmonary embolism and pneumonia.
29	III	V.S.	Cured C.S.F. Died very severe pneumonia later.
92	II	V.S.	Died of double lobar pneumonia.
181	II	V.S.	Died of septic broncho-pneumonia.
182	II	V.S.	,, ,, ,, ,,
183	II	V.S.	Died of septic broncho-pneumonia, with acute œdema of lungs.
187	II	V.S.	Acute cardiac dilation with pulmonary embolism.
188	II	V.S.	Double influenzal pneumonia and jaundice.
191	I	S.	Died later with diphtheria and acute dilation of heart.
204	II	V.S.	Had a very virulent influenza at same time.
205	II	V.S.	,, ,, ,, ,,
206	II	V.S.	,, ,, ,, ,,
207	II	V.S.	,, ,, ,, ,,
208	II	V.S.	,, ,, ,, ,,
210	I	V.S.	,, ,, ,, ,,

Note.—The last six cases occurred during an epidemic in which other patients died of influenza alone, and all occurred within a week; they were practically fulminant, and each had only one dose of serum. No. 187 is the only case I had doubts about including in this table, but the pulmonary embolism led me to do so.

V.S. = Very severe C.S.F.

Tables IV and V contain respectively a summary of cases which the reporters did not expect to recover from the first, and those in which the action of the M.R.C. serum attracted special attention. Table IV does not seem to me to require any remark, as such cases are bound to occur; some recovered and some ended fatally. On

the whole, perhaps, the expectation of a fatal ending proved not to be justified ; but whether these good results were due to the action of serum in all these cases is not clear. As this table is of so little interest I have omitted it.

With regard to Table V, in which the beneficial action of the serum was specially marked, a more than sufficient number of cases in which the action was of interest was given in the ' first 90 cases ', and it would be easy to elaborate a similar table for the complete series, of several times the length. As I have no wish to make this paper appear like an elaborate patent medicine advertisement I refrain from doing so. I have, however, taken one or two examples of the remarks that have been made in about 100 of the cases and here give them.

28. Type II. Fulminating, thought hopeless ; slow improvement after pooled I and II, but remarkable after Type II serum. Cured.

32. Type III. Very severe. No improvement with pooled serum (only I and II) : one dose of III serum then cured patient. Paralysis of arm also disappeared. Reporter states that real improvement began in his other cases only when the correct monotypical serum was given.

69. Type III. Serious case, reporter says wrongly diagnosed at first and ' bungled '. First dose of III serum ' temp. over 100° F., dropped finally and completely, and appearance of patient literally transformed.' Cured.

114. Type IV. Extremely severe case, coma, widespread purpuric rash, &c. Considered quite hopeless. After three doses IV serum became sensible, regained memory with a good cure at end.

141. Type I. Very severe case ; after five doses pooled serum, three doses of Type I serum given and then improvement rapid. Reporter says recovery was ' most striking '.

222. Type III. Very severe case, showing nearly all the worst signs. ' After two doses pooled (I and II) he got his Type III serum ; marked improvement at once. Temp. dropped ; he became quite rational, and made good recovery with two doses.'

We now come to Table VI, in my opinion one of the most important tables shown ; it is short but full of interest.

TABLE VI.

	Cases.	*Deaths.*	*Mortality. percentage.*
Type I	65	6	9·23
„ II	104	41	39·42
„ III	28	7	25·0
„ IV	4	3	75·0
Untyped	48	22	45·8

A similar table to VI, but confined to children under 15 years of age is also here shown.

TABLE VII.

	Cases.	*Deaths.*	*Mortality. percentage.*
Type I	5	0	..
„ II	10	6	60·0
„ III	8	3	37·5
„ IV	1	1	100·0
Untyped	5	4	80·0

The above children's cases are also included in Table VI.

It will be noticed in Table VI that the death rate in cases due to Type I is still very low—under 10 per cent. Considering that out of the 65 cases 48 were reported as fulminant, very severe, or severe, the

percentage of recoveries is excellent. This fully bears out the conclusion previously arrived at that the Type I serum was of great value ; moreover, it is specially interesting in that it is a clinical confirmation of the results of the experimental testing, in which the mice injected with the toxin of Type I almost invariably recovered when treated with Type I serum, whereas the control mice as frequently died.

The same remark, but in an inverse direction, unfortunately, can be made about Type II serum. As previously mentioned, the percentage of deaths is nearly 40 per cent.

Whatever influence influenza or other adverse factors may have had on the increased mortality, these cannot be alleged as the cause of the failure with regard to any particular type of serum. No attempt has been made to analyse whether the Type II cases have been proportionately more affected by influenza than the other types, chiefly because all the reporters do not give sufficient information on this point. It is true there were one or two localized epidemics of Type II, following outbreaks of influenza, but the available data seem to indicate that the types were fairly impartially distributed among the influenzal cases.

Dr. Griffith has been successful in producing a potent serum against Type I endotoxin, and almost equally so with Types III and IV, but the antiendotoxin for Type II still proves much more difficult to produce satisfactorily. Although certain batches of serum prepared against this coccus have proved to be reasonably potent, experimentally, he has not, even yet, been able regularly to produce such a strong serum against this type as against Type I. So much was this the case that, owing to the large demands for Type II serum, Colonel Gordon was obliged to pass this serum for issue when it contained some antiendotoxin, though not to the extent necessary completely to satisfy the laboratory test. The dose of this serum was at the same time, therefore, raised to 30 c.c. from 25 c.c., so as to afford more protection, and it was advised to give the dose more frequently where possible.

This lack of potency in Type II serum is doubtless the reason for the Type II mortality being so much higher than the Type I, although, if previous experience be trustworthy, the Type II coccus is less virulent than Type I for the human subject in the majority of cases.

A close parallel to this unfortunate failure to produce a potent serum against the Type II coccus is afforded by the reports, lately published, on the antipneumococcic serum. In pneumonia, provided the patient be infected with a Type I pneumococcus, his chance of being cured by the appropriate serum will be good, whereas the sera for the three other types have not proved to be of corresponding potency.

With regard to the cases due to Types III and IV, Type IV has only four cases, and therefore any figures deduced therefrom are of little value, and the same remark, though in a lesser degree, applies to Type III, with its 28 cases.

There is a strong probability, however, that many of the Type III and IV cases were diagnosed as such rather late, and did not have the

advantage of early administration of their proper monotypical serum, the pooled serum, of course, containing no specific antiendotoxin for these types. The remarks by many of the reporters that these cases showed little if any improvement before the proper monotypical serum was given bear out this supposition. There were, of course, a few cases which recovered after the administration of pooled serum alone, and it is yet to be proved that this pooled I and II serum has no beneficial effect at all against Types III and IV endotoxin, though such effect is undoubtedly only of a minor character. To those familiar with the peculiarities of the disease, these recoveries will occasion little surprise.

The importance of Table VI is twofold. In the first place it provides a valuable clinical corroboration of the experimental work on which the principle of administering monotypical serum is based ; for, as is clearly seen, the clinical success, or failure, is closely parallel to the result of the experimental testing of the serum, as described by Colonel Gordon. The success of the Type I serum alone should be a valuable stimulus to those wishing to continue to elaborate further treatment on these lines in this or similar diseases.

In the second place, this table shows the value of an attempt to ' type ' the cases as early as possible. When it is observed that 48 cases which were ' untyped ' show a mortality of over 45 per cent., while the combined mortality from Types I and II (despite the lack of potency in Type II serum) only amounts to 26 per cent., it would seem obvious that a promising step has been made in using this method of diagnosis and treatment. It will be well here to recall that Flexner, from a large number of cases, found the mortality, in cases untreated by serum, to be from 65–80 per cent., while Dopter shows a mortality of 65 per cent. with a similar lack of serum treatment in an epidemic he observed in France.

Table VII, shown after Table VI, gives the type mortality of 29 children included in Table VI. Children would appear to have a higher mortality than adults, though the numbers are too small to make any inference reliable. It may possibly be partly due to the difficulties of serum treatment in children and partly to a fact, which one reporter significantly mentions, that ' it is difficult to get some of the "honoraries" to allow daily lumbar puncture and injection of serum to be performed on their patients '. This handicap is, however, unfortunately, not only confined to the treatment of children.

Table VIII is designed to show the mortality of the disease according to the day on which serum was first administered. It would be perhaps more convincing if a larger number of cases were available for each day. If the days, however, be taken in groups, the results become more even and the deductions more reliable.

This table (p. 185) is of interest as showing the importance of an early administration of serum. This is, of course, no more than one would expect on the analogy of diphtheria serum, though the results are not so well marked. Incidentally it shows that I was justified in my choice of the sixth day as the dividing line between satisfactory and unsatisfactory conditions for a test of the serum.

TABLE VIII.

Mortality, according to the day of disease on which the first dose of serum was administered, of cases appearing in Groups A and B (Table I).

Day of First Dose.	*Cases.*	*Deaths.*	*Mortality per Cent.*		
1	10	3	30·0		
2	44	7	15·9	24·74	
3	43	14	32·55		25·00
4	21	4	19·04		
5	18	5	27·8	25·45	
6	16	5	33·4		
7	9	6	66·67		
8	10	3	30·0		
9	2	1	50·0	43·18	
10 and after	23	9	39·13		
Total	196	57			

There were also five cases, the day of first dose being unknown.

Table IX (p. 186), which is a comparison of the mortalities shown by medical officers reporting, may seem at first sight a somewhat unnecessary table. The greatly differing mortalities, however, impressed me profoundly, and, as they were all using the same serum, I felt that it might be possible, by analysing the results, to arrive at some cause which might account for this variation.

Accordingly I selected a number of reporters who each had a fair number of cases—18 reporters in all, averaging 11·1 cases apiece, or 201 cases. These must not be confused with the 201 cases in Table I, but are taken from this table and Table II, the correspondence in numbers being a mere coincidence. It was decided to place these reporters together in groups so that the results obtained by individuals could not possibly be identified, and to average their figures. The groups were so arranged as to have something like the same number of cases in each, the individual reporters being arranged in an ascending sequence of mortality, the two lowest having no deaths, while the two highest had 66·6 and 100 per cent. respectively. It turned out that the first group contained six reporters, having each a mortality below 20 per cent.; the second group five, with mortalities between 20 and 40 per cent.; and the last, with seven reporters and mortalities from 40 to 100 per cent. To summarize :

Group 1, 62 cases, 7 deaths = 11·29 mortality
,, 2, 74 ,, 20 ,, 27·02 ,,
,, 3, 65 ,, 38 ,, 58·46 ,,

The above table shows the reason which persuaded me to go farther.

On looking through the various reports no great and outstanding difference in methods of treatment was apparent, though perhaps doggedness and persistence were features of the reporters in Group 1, while a certain indecision in giving the serum, coupled perhaps with bad luck in not getting cases quite so early, or at any rate not giving the serum so early, seemed to distinguish those in the latter groups.

It seemed possible there might be some other reason which could be demonstrated by figures, in preference to trusting to impressions incapable of proof, especially as these differences of method did not appear to be sufficiently marked to be the only explanation.

TABLE IX.

Name.	No. of Cases.	Severe.	Moderate.	Proportion S. M.	Deaths.	Types. I.	II.	III.	IV.	Untyped.	Average day of First Dose.	Do. for Death.	Types in Cases which Died. I.	II.	III.	IV.	Untyped.	Death-rate per Cent.
Group 1 . .	62	44	18	2·44:1	7	18	30	11	..	3	4·1	7·0	..	7	..	..	..	11·29
Group 2 . .	74	50	24	2·09:1	20	31	24	8	2	9	5·0	4·9	2	11	2	1	4	27·02
Group 3 . .	65	44	21	2·09:1	38	10	32	9	1	13	6·0	5·1	5	21	6	1	5	58·46

TABLE X.

MORTALITY FROM CEREBROSPINAL FEVER AMONG THE CIVILIAN POPULATION IN ENGLAND AND WALES, INCLUDING LONDON, 1914–18.

Year.	All Ages. Cases.	Deaths.	Mortality. (Per cent.)	Males, 20–30. Cases.	Deaths.	Mortality. (Per cent.)
1914	300	206	68·7	9	4	44·4
1915	2,343	1,521	64·9	127	80	63·0
1916	1,278	838	65·6	45	23	51·1
1917	1,385	906	65·4	48	31	64·5
1918	715	484	67·6	12	7	58·3
Total	6,021	3,955	65·6	241	145	60·1

This table is compiled from the Reports of the Medical Officer, Local Government Board, 1917–18 and 1918–19, and is inserted for comparison with the figures in this analysis. It would appear from this table that the mortality from cerebrospinal fever among the civil population was fairly constant round 65 per cent., whereas the mortality among all those treated with M.R.C. serum (Tables I and II) was 31·72 per cent.

I first divided the cases into ' severe ' and ' moderate ', according to the reporters' own descriptions. The result of this was that the proportion of severe to moderate was 2·19 severe or fulminating, to 1 moderate or mild. On reference to the table it will be seen that the proportion in the first group is 2·44 to 1, while in the other two it is 2·09 to 1 in each case ; this factor, therefore, does not elucidate the matter : the trend is rather in the opposite direction.

The next possibility was that a large proportion of the child cases happen to fall into the last group, and that their known higher rate of mortality might have had a bad effect ; however, on removing them from the group, the mortality from the remaining adult cases was raised from 58·6 to 60·0 per cent. This explanation, therefore, is also untenable.

A further factor, the proportion of Type II cases to each reporter, seemed worthy of investigation, as we have seen that this type has by far the greatest mortality of the two commoner types, while the Types III and IV form such a small proportion of the total cases that their influence is practically negligible. I accordingly took out the number of each type in each group with the result that Group 1 had 30 Type II's to 32 of the other types ; Group 2 had 24 Type II's to 50 of the other types, and Group 3 had 32 Type II's to 33 of the other types. It is obvious, then, that the first and last groups are about equally proportioned, while the second group with a mortality of about 2½ times the first had only about half its proportion of Type II cases.

It will, therefore, be seen that these first three possibilities only go to make the explanation more difficult, as the first group labours under all the disadvantages in question to a greater extent than the others.

In the ' distribution of types ' portion of the table it will be seen that the first group had only three untyped cases, the second 9, and the third 13. In the first group all these three recovered, but in the other groups nine deaths occurred in these cases. This looked as if it might throw some light on the problem, but if the untyped cases dying be eliminated from each group, it only reduces the mortality to 22·9 and 55 per cent. respectively.

I then investigated the cases according to the earliest day of the disease on which serum was given. In Group 1 the average proved to be the 4·1 day, in Group 2 the fifth day, and in Group 3 the sixth day. This, at least, looks as if the day of disease on which the patient receives his first dose might be an important factor in explaining matters and confirming the results obtained in Table VIII by a different method. I went on to take out similar figures for the cases dying. It was found that the cases which died in the first group received their first dose on the average on the seventh day ; in the second group, it was the 4·9th day ; and in the last group on the 5·1th day. This at first sight would appear anomalous, but it indicates (if there be anything beyond coincidence in it at all) that the officers in the first group were capable of saving the patients who got their first dose up to the seventh day, whereas the officers in the other groups could only do so provided they could first treat their cases before the fifth day.

However interesting these speculations may be, it does not appear

justifiable to form definite conclusions on any one of them; but I think it may perhaps be fair to conclude that an explanation is probably to be found in a combination of several of them, together with the 'personal equation' of the officer in charge of the treatment. In short, my first impression, that persistence and doggedness as against indecision and procrastination are big factors in the explanation, still stands, and I have failed to find any other outstanding reason to account for the great variation in mortality.

It must not be forgotten that many of the cases were treated under grave disadvantages. Only a few of the reporters were responsible for the treatment, or, at least, not after the first dose of serum, which was usually given when the case was first seen. The cases were then generally transferred to another officer, often with a trying journey to reach him. This is bad policy and tends to irregularity in treatment, as is often brought out in the reports. That which strikes me most forcibly, though I am precluded from actually proving it by my method of dealing with this table in order to avoid personal distinctions, is that the man who has quickly identified the type of invading meningococcus and has been able to treat the case from start to finish without interruption and with unflagging attention has been able to show the most successful results.

24. The Significance of the Serological Types of the Meningococcus.

The four types of the meningococcus have been found to breed true alike in carriers, in patients who recover from cerebrospinal fever and continue to be carriers, or in cultures kept up on artificial media. During the four years that have elapsed since the types of meningococcus concerned in the outbreak were defined, the serological characters of these micro-organisms have been under constant investigation at the Central Laboratory. This experience has shown that some meningococci are of better agglutinogenic value than others, and that on repeated subculture meningococci may deteriorate in agglutinogenic capacity, or may become hypersensitive to agglutination and take to agglutinating with normal serum; but it has revealed no alteration in the more fundamental property of these micro-organisms of combining with their own type agglutinin, and it has brought to light no certain instance of the mutation of one type into another.[1] During the period under review four annual recurrences of cerebrospinal fever among troops in training have provided a very searching and practical test of the efficiency of this serological classification of meningococci into four types, with the result that not only has its accuracy and comprehensiveness been fully established, but also the general opinion based on the evidence obtained in 1915 as to the relative frequency of each of these types respectively.

The careful and independent observations of Flack, Tulloch, Embleton, and others have demonstrated that as a rule a case of cerebrospinal fever or a chronic carrier harbours only a single type of the meningococcus and that this type remains constant throughout the period of carrying. In case of carriers herded together in

[1] No meningococcus, of course, was adopted for preparing type agglutinin until its identity with the specimen previously in use for this purpose had been proved by the absorption test.

isolation this rule is not inviolable; Fildes has given particulars of some instances in which he observed a change of type in the nasopharynx of a carrier—the result apparently of cross-infection—and Bassett-Smith and Lynch have also observed such change in carriers. From the very large number of observations that have been made on troops, however, and particularly from the comprehensive studies carried out by Embleton, there can be no doubt whatever that as a rule both in cases of cerebrospinal fever, and in carriers, the infection is strictly monotypical.

With regard to the relative capacity of each of the four defined types of the meningococcus to set up cerebrospinal fever, the fact that 80 per cent. of the cases examined have yielded specimens either of Type I or of Type II indicates that these two groups include the majority of meningococci of pathogenic significance to man. In his investigation of cerebrospinal fever among new entries into the Navy between 1916–17, Fildes found that although Type II was four times as common in the nasopharynx of these men as Type I, Type I was twice as frequent as Type II in the cases. He inferred that meningococci belonging to Type I are more successful in producing disease than those belonging to Type II. This view is in accordance with the fact that during the rising wave of the severe outbreak among troops in training in the early months of 1915 Type I was especially prominent. A number of severe outbreaks, however, have occurred in which Type II has been chiefly concerned, and in the aggregate of meningococci examined at the Central Laboratory during the four years 1916–19 Type II has been found on the whole to excel Type I in frequency, though the difference between them is not great. It may here be mentioned that according to the French, Type I of our outbreak is identical with the true meningococcus, and Type II with the parameningococcus of Dopter; while the Americans regard Type II as the 'normal' meningococcus because it is more common, and Type I as the parameningococcus. This confusion with regard to the parameningococcus is to be regretted; to avoid mistakes it would seem advisable to restrict that term to Type II.

With the exception of Newcastle, to which locality reference has been made previously, Type III has not been found in any large proportion of cases during an outbreak. Type IV also has only been observed with any frequency in a single outbreak, and on the whole has been the rarest of all the types in military cases. It is of interest to note therefore that Fildes, in whose experience Type IV would seem to be rather more common than in ours, found that this type was more persistent than the others in his carriers, and further that occasionally it was apt to replace other meningococci in the nasopharynx. On cultivating a mixture of Types I, II, and IV *in vitro* also he found that whereas Types I and II were mutually tolerant of each other's presence, and grew side by side with equal vigour, Type IV displaced Types I and II from the mixed culture entirely. Notwithstanding this capacity to overcome other meningococci observed by Fildes, Type IV has been the least common of all meningococcus types in military cases.

With regard to the intensity of the disease in individual cases according to the type of the infecting coccus, Kennedy and Worster

Drought drew attention in 1917 to the fact that in their experience cases infected by Type I were far more fatal than those infected by Type II, and this would seem to correspond with the general experience of others up to that time. Fulminating cases, however, have been reported with all the types, and during the later stages of the outbreak a number of severe cases with Type II have occurred. When the mortality tables over the whole period are available it is hoped that more precise information will be forthcoming with regard to the death-rates from individual serological types of the meningococcus.

While the specific agglutinin evoked by a meningococcus in the rabbit has been proved by the absorption test to be monotypical, scrutiny of the co-agglutinins also present in such serum indicates that Types I and II are related, and also Types II and IV. For this reason it is possible to divide meningococci broadly into two main groups, and to regard Types III and IV as specialized offshoots of Types I and II respectively. A hypothesis, however, which is based upon affinities indicated solely by co-agglutinins is open to the criticism that it should include gonococci as well, and, as will be seen later, it would seem wiser to avoid speculation concerning the genealogy of meningococci in their pathogenic relation to man until we have more information than is at present available concerning the pathogenic attributes of these cocci, and also concerning the internal and external relations of the group.

The problem of the relationship between the four serological types of the meningococcus was raised in a previous report and may be formulated as follows : Are these serological types mere temporary variants of one and the same micro-organism or are the distinctions between them fixed and permanent ? This question is of such basic importance in its bearing on present conceptions concerning the etiology of cerebrospinal fever that it is desirable to review the available evidence on the matter before proceeding further.

It is necessary in the first place to recapitulate. Investigation of their antigenic capacity for the rabbit proved that meningococci concerned in the outbreak belonged to one or other of four specifically distinct agglutinogenic types. Confirmatory evidence of this diversity was afforded by the observation that while a given meningococcus was found to combine with the specific agglutinin of its own type *in vitro*, it exhibited no such affinity for the specific agglutinins of the other three types of meningococcus. Both from the action of the agglutinin on the coccus therefore, and also from that of the coccus on the agglutinin, it was evident that meningococci concerned in the outbreak belonged to one or other of four distinct antigenic races. As the investigation of over 500 further specimens of meningococcus has failed to reveal any further types it may be taken that, so far as agglutinin can show, diversity among meningococci is limited to the four types in question.

With agglutinin still as guide, evidence was next sought from another direction. The argument underlying the present tests was as follows. If these four types of meningococcus are transient variants of one and the same micro-organism, then in the case of a rabbit under preparation with a meningococcus of a known type,

and already in course of elaborating agglutinin to that type, the injection of a meningococcus of a different type should merely increase the production of agglutinin which the rabbit is already in course of elaborating. If, on the other hand, the difference between the four types is fixed and specific, then on the super-imposition of a heterologous type of meningococcus the primary agglutinin, so far from being increased, should show its normal decline, and a new agglutinin specific for meningococcus No. 2 should make its appearance in the rabbit's blood. On putting the matter to the test of experiment and testing the serum of each rabbit not only with the agglutinin test, but also with the absorption test, it was found that in each case when a meningococcus of a heterologous type was injected, after a suitable interval a new agglutinin specific for the secondary antigen and previously absent made its appearance in the rabbit's blood. The agglutinin for the primary antigen also underwent its normal decline in these experiments with one exception—namely, when Type III was superimposed on Type I, when coincident with the appearance of the new agglutinin for Type III in the rabbit's blood the agglutinin for Type I underwent a rise. Other observations, however, indicated that this increase of the primary agglutinin for Type I was due not to increase in the specific agglutinin for that type, but to the action of a co-agglutinin known to be evoked by Type III for Type I. These super-imposition tests therefore afforded a striking confirmation of the antigenic specificity of the four types of the meningococcus.

Now the antigenic property of the meningococcus is of course by no means limited to the production of agglutinin. For the purpose of specific treatment it was of the utmost importance to determine, if possible, whether immunity follows the lines indicated by the study of agglutinin, or not. Accordingly an attempt was next made to determine whether prophylactic inoculation with a given type of meningococcus is protective, and if so whether such protective action is limited to the type injected, or if it extends also to other types of the meningococcus. A group of rabbits was prepared against Type I, another against Type II, a third against Type III, and a fourth group against Type IV. After six weeks or so of preparation by the intravenous administration of first killed and then living cultures, one rabbit of each group and a control normal rabbit were saturated by the intermittent intravenous administration of living cultures of Type I, another set in the same way with Type II, and further sets with Types III and IV. As a result it was found that in the case of Types II, III, and IV, the sole rabbit that survived was the one that had been prepared against the particular coccus with which the saturation test had been performed. In the case of these types therefore the immunity appeared to be mainly of a univalent character. In the case of Type I, however, the homologous rabbit, so far from being protected, was found in spite of the prophylactic inoculation to be, if anything, less resistant than a normal animal. The whole series of experiments was repeated and a similar result obtained. So far as they went, therefore, these resistance tests indicated that in at any rate three of the four types the immunity produced by prophylactic inoculation was mainly univalent in character. In the

case of Type I, however, it was apparently more difficult to protect the animal.

The conclusion drawn from these experiments and observations which were described in greater detail in the report of 1917, was as follows:

'The experience related, experimental and other, demonstrates that, under laboratory and epidemic conditions alike, the four serological types of meningococcus breed true, and preserve their distinctive character when propagated. In spite of their general similarity and close relationship, these four meningococci behave as specifically distinct micro-organisms. According to present evidence, then, the current outbreak of cerebrospinal fever is due not to transient and unstable variants of a single micro-organism, but to a group of individual species of meningococcus of which the first two between them so far have accounted for over 80 per cent. of the cases. But while the meningococci in question exhibit a well-marked fixity as regards their distinctive serological characters under the conditions observed, it must not be assumed that the limit of their individual variation has yet been defined.'

This conclusion, which seemed to be the logical outcome of the result of the observations and experiments related, has met with a certain amount of adverse criticism. It would seem that the existence of serological diversity among micro-organisms presenting the morphological cultural and fermentative characters of meningococcus and recovered from the cerebrospinal fluid of cases of cerebrospinal fever is not disputed, and also the existence of two different antigenic races among them. Apparently it is the validity of Types III and IV that is the main object of attack, and above all the biological conceptions of these critics appears to have been somewhat shocked by the application of the term specific to each of the four serological types of the meningococcus respectively.

Now the existence of four distinct serological types of the meningococcus has been a matter of almost daily observation in the Central C.S.F. Laboratory for a period of some four years past, and out of the 526 meningococci from the cerebrospinal fluid of cases examined during this time no less than 518 have been found to qualify serologically as examples of one or other of these types. The actual number of these meningococci classed as specimens of Types III or IV was 69 and 36 respectively, amounting in all to 105 specimens, or about one-fifth of the total number of meningococci received. As a matter of practical and repeated experience, therefore, Types III and IV have a very real existence indeed, since without them no less than 20 per cent. of the meningococci, isolated chiefly from soldiers suffering from cerebrospinal fever, and examined at the Central C.S.F. Laboratory, could not have been serologically identified.

In order to check this serological differentiation of meningococci into four groups, and for the purpose of obtaining more information concerning the relations of meningococci included under Type II, Major W. J. Tulloch independently went over the ground once more and submitted specimens of meningococci isolated from the cerebrospinal fluid of 107 *further* cases of cerebrospinal fever to a most careful and thorough scrutiny with the agglutination and absorp-

tion test. Three of these cocci died before their investigation was complete ; of the remaining 104 cocci no less than 101 were found by Major Tulloch to be relegated by the absorption test to one or other of the four types ; the distribution being as follows :—Type I, 30 specimens ; II, 41 ; III, 13 ; and IV 17 specimens. He concluded that the sub-division of meningococci occurring in the outbreak into four serologically distinct groups was fully justified.

Major Tulloch found that Type II is a more complex group than the others, and far more difficult to study. Not only is satisfactory agglutinin more difficult to prepare in case of many specimens of Type II, but members of this group may become ultra-specific serologically after they have been growing on artificial media for some time. By preparing a separate serum against each of a number of specimens of Type II, however, and by then carrying out complete cross-absorption tests he found that no less than 41 out of 44 specimens of meningococcus provisionally classed as Type II as the result of preliminary agglutination were definitely included within Type II by the absorption test. Major Tulloch's observations established also the important point that an agglutinating serum prepared against a sufficiently representative specimen of Type II will include the great majority of Type II cocci, and as a direct result of this observation it has been the practice at the Central Laboratory since Major Tulloch's investigation to take special steps to ensure that the agglutinating serum sent out for identifying specimens of Type II meningococcus shall be as catholic as possible for the group, as may be seen from Major Bell's article on the preparation of agglutinating serum.

The serological differentiation of meningococci into four groups, however, is not limited to observations carried out on military cases. When it was found that practically all the meningococci occurring in cases among troops could be definitely included in one or other of these four groups, Lieut.-Commander Fildes, being engaged in a study of cerebrospinal fever among new entries into the Navy, sent for a culture of each of the four types of meningococcus, and entirely from his own observations and experience, and with agglutinating serum prepared by himself with cocci isolated from his cases, fully confirmed the existence of these four serological types among meningococci occurring in his cases and carriers. His conclusions were as follows :

' Gordon's statement that practically all meningococci capable of producing cerebrospinal fever are found to belong to one of his four groups is confirmed by our results. It therefore follows that an unknown coccus from the throat or elsewhere which does not belong to one of these four groups is not likely to be a pathogenic meningococcus. This rule may be taken to be invariable for practical purposes.'[1]

At an early stage of the recent outbreak the Medical Department of the Local Government Board, while admitting the diagnostic value of the presence of meningococci in the cerebrospinal fluid of cases of meningitis, adopted an attitude of profound scepticism with regard to

[1] In a paper published while this report is in the press Fildes recedes somewhat from this position because of difficulty in placing recent strains. Such strains from sporadic cases have usually in our experience proved to be specimens of Type II. They are identified by certain Type II sera from which alone they remove the specific agglutinin.

the value of nasopharyngeal swabbing for dealing with outbreaks of cerebrospinal fever, and this attitude found support in the result of the researches of the Board's pathologists upon the matter. Serological investigation of strains of meningococci derived from cases among the civil population, and of meningococcus-like organisms from the nasopharynx of non-contacts, led them to conclude that while two main races of meningococci could be broadly distingiushed, the existence of intermediate serological strains invalidated the identification of the meningococcus in the nasopharynx by serological means. The conclusion arrived at was that for the purpose of identifying the meningococcus in the nasopharynx of carriers it was advisable to rely upon an examination of the morphological cultural and fermentative characters alone of this micro-organism.

The methods adopted in the army for dealing with cerebrospinal fever have been fully described in the present report. When the disease broke out among recruits in training during 1915, carriers were perforce identified by examination of the morphological, cultural, and fermentative characters alone of the suspect cocci. Meanwhile meningococci occurring in the cerebrospinal fluid of the cases were investigated serologically and found to conform to one or other of four distinct serological types. On now submitting micro-organisms isolated from the nasopharynx of contacts, and presenting the morphological cultural and fermentative characters of meningococcus, to serological investigation with the four sera found to include the types involved in the outbreak, no less than a third of these pharyngococci were found to be differentiated thereby from the meningococcus. Because it had proved superior in accuracy, therefore, from that time onward chief reliance was placed on the serological method of identifying the meningococcus. The possible occurrence of new types was provided for by keeping a continual watch on the serological character of meningococci occurring in the cerebrospinal fluid of cases. As a result, altogether over 500 meningococci obtained from the cerebrospinal fluid of cases have been examined, and over 98 per cent. of these have been found to conform to one or other of the four types.

The group of nasopharyngeal micro-organisms presenting the general morphological, cultural, and fermentative characters of meningococcus, but failing to conform serologically to any of the four defined types of that micro-organism has been investigated further by Major Tulloch, whose research on these pharyngococci is reported in a previous section (pp. 111–127). As might, perhaps, have been expected, he found that they comprised a large number of serologically distinct races. The significance of these pharyngococci is at present a matter of speculation. It is possible that some of them may be of importance in relation to catarrhal conditions. That they have any pathogenic significance whatever in relation to epidemic meningitis has still to be demonstrated. The whole of the evidence obtained at the Central C.S.F. Laboratory during four and a half years' continuous study of cerebrospinal fever in the army is uniformly against it.

The finding of meningococcus-like organisms in the nasopharynx of 10 per cent. of non-contacts in populous areas by the pathologists of the Local Government Board leaves one in doubt as to exactly how

many of these cocci were serologically identical with one or other of the four types of the meningococcus identified in military cases. If a reduction of 30 per cent. be made for inagglutinable strains, the percentage works out at seven. The extensive and continuous studies of the carrier rate described by Captain Glover would seem to supply the perspective necessary for interpreting these observations. It will be noted that a carrier rate of 20 per cent. is regarded as the danger line in the army.

It has been pointed out previously that individual strains of the meningococcus differ in agglutinogenic power, and as Major Bell explains in his article on agglutinin, it has been the practice at the Central Laboratory, particularly in the case of Type II, to test the agglutinogenic capacity of a number of strains before selecting one to employ for preparing agglutinating serum for routine purposes.

In a recent paper on the allocation of meningococci to their type by agglutination, Major A. G. Gibson and Mrs. Ludlow Hewitt describe certain difficulties they have had, and also the result of some agglutinogenic experiments on rabbits and on man in which they failed to find evidence of type specificity. They instance a few points in which the technique used by them differed from that advised in the pamphlet issued from the Central C.S.F. Laboratory, which alterations may or may not have contributed to the difficulties met with. Some of the charts given by them, however, illustrate a serious source of fallacy, namely, their omission to determine the actual end-point of the agglutination in a considerable proportion of those tests. Now this is hardly the fault of the method, but of its application. When two sera such as II and IV both agglutinate a coccus, it is absolutely necessary to determine the end-point to each in order to discover the type to which the coccus belongs. Our experience at the Central C.S.F. Laboratory has invariably been that the coccus agglutinates highest with the serum of its own type. They did not apply the absorption test which almost certainly would have solved their difficulty in typing some of these cocci. Meningococci differ in agglutinogenic power, and they appear to have been unfortunate with some of the specimens which they injected into rabbits. The experiments in which they injected human beings subcutaneously appear to have given very poor results as regards agglutinin of any kind. The fact that they found some agglutinin for another type than that injected goes for very little. The subcutaneous route is not to be compared with the intravenous one for producing agglutinin in animals, and man is not likely to be an exception. From the observations of Fildes, even the blood of persons affected by cerebrospinal fever may fail to show agglutinin for the meningococcus.

The evidence of apparent pulmonary thrombosis found by Major Gibson histologically in rabbits that succumbed to saturation with the meningococcus, so far from invalidating the immunity tests described previously, has received a very different explanation from the recent work of Coca, who has shown that similar histological lesions present in the lungs of animals that succumb to anaphylaxis are to be attributed, not to embolism, but to occlusion of the pulmonary artery from spasm of its muscular coat brought about by toxic agency. The lesions in question, therefore, suggest a relative

inability on the part of the animal to withstand the endotoxin of the meningococcus.

Professor Walker Hall has carried out some interesting observations on the serological characters of meningococci when freshly isolated from the cerebrospinal fluid of the same case from day to day, and has found evidence of considerable variation and apparently of change of type. Unfortunately it has not been possible hitherto for these experiments to be repeated on a large scale. The serological characters of different colonies from primary plates made from the cerebrospinal fluid of patients suffering from cerebrospinal fever, however, have been tested in 26 cases, with the result that all colonies from the same case were invariably found to give practically the same serological reading; and Colonel Flack examined in a similar fashion cultures obtained by successive punctures in several cases of cerebrospinal fever without finding any evidence of change of type. A point that makes one a little sceptical of Professor Walker Hall's readings is that the agglutinating serum sent out from the Central Laboratory for identifying the meningococcus by agglutination, and used by him, is not necessarily the best for absorption tests. For the latter purpose it has been the practice throughout for each investigator to prepare his own agglutinating sera and to select those that give the best results. It would be interesting to know if Professor Walker Hall kept the meningococci from the same case which he found to give different readings when freshly isolated, and tested them over again when they had been in culture for some time. It is difficult to reconcile these observations with those of others on the identity of the type of meningococcus in the nasopharynx and cerebrospinal fluid of the same patient, and with the persistence with which cases and carriers alike have been found to maintain the type of meningococcus carried by them unchanged. At the same time it is conceivable that the serological characters of a meningococcus—especially an example of Type II—may undergo some modification in the human tissues. A great deal of interaction must occur there between the coccus and its host as a result of which possibly the autolytic tendency of the coccus increases so that, when freshly isolated, groups for heterologous types may be liberated (?). The field opened up by Professor Walker Hall's observations is of much interest, and further study of this matter is desirable.

A sound criticism with regard to the conclusion arrived at in the report of 1917—a point, moreover, that seems to have escaped the attention of the critics—is that since agglutinin is only one of a series of specific antibodies which the living body generates in defence against the meningococcus, it is advisable to suspend judgement on the subject of the relations of the types of that micro-organism to one another until they have been investigated from the point of view of antibodies other than agglutinin. It was partly to meet this criticism, but chiefly with a view to acquiring information for the purpose of obtaining therapeutic serum of higher efficacy, that the studies of these antibodies were undertaken which have been described in detail in a preceding portion of this report. The result of these observations so far as they bear on the present problem are as follows:

Complement-Fixing Antibodies. This investigation was carried out on his own initiative by Major Bell with the valuable assistance of Miss Iris Harmer. When applied to the four monotypical agglutinating sera prepared by Major Bell from rabbits for identification of the meningococcus, or to the monotypical sera prepared by Dr. Stanley Griffith from horses for therapeutic use, the complement-fixing test was found to be more delicate than the agglutination test, and to confirm the results of the latter with regard to the specificity of the four types of meningococcus. It would appear, however, that there is no fixed relationship between the two processes of agglutination and complement-fixation.

Major Bell next proceeded to examine the ability of serum from cases of cerebrospinal fever, from control cases, and from meningococcus carriers respectively, to fix complement in the presence of each of the four types of meningococcus. The serum from the majority of the control cases showed a relative absence of complement-fixing antibodies to the four types. The complement-fixing antibodies were found to be considerably increased in the serum of patients suffering from cerebrospinal fever. Although the results as between types were not clean cut, an indication could be obtained in a good proportion of the cases as to the type of the infecting coccus, and since complement-fixing antibodies may be present as early as the fifth day from the onset, the method, as Major Bell points out, is available within limits for determining the type of meningococcus infecting the case. Serum from chronic meningococcus carriers was found to contain more complement-fixing antibodies for the meningococcus than serum from the control cases, but these antibodies were on the whole much less plentiful than in the serum of patients suffering from cerebrospinal fever. Occasionally the test gave an indication as to the type of the infecting meningococcus, but more often such indication was lacking.

Opsonin. Up to the present difficulties of technique—in spite of a persevering attempt by Major Tulloch to overcome them—have prevented a decision being reached with regard to the specificity, or otherwise, of the opsonin formed against individual types of the meningococcus.

Lysin. The studies of this antibody recorded in a preceding section indicate that the lysin for Type I is distinct from that for Type II, but the investigation had to be abandoned before sufficient evidence had been obtained to permit of a final conclusion, and before Types III or IV could be submitted to scrutiny in the same way. It is of interest, therefore, to recall that Dopter, by his *in vivo* test described in the section on lysin, found evidence of the presence of specific lysin for the meningococcus and for each of his three parameningococci respectively. He also found evidence of the presence of co-lysins.

Antiendotoxin. The evidence obtained so far indicates that the types of the meningococcus defined by means of agglutinin are distinct also as regards their endotoxin. Additional investigation, however, also showed that the serological types as defined by agglutinin are to be subdivided still farther by reason of the important fact that meningococci indistinguishable from one another by agglutination and by the absorption of agglutinin test may neverthe-

less differ *inter se* in respect of endotoxin. Preliminary observations indicate that within Type I more than one endotoxin obtains, and within Type II at least three.

It would appear, therefore, that the meningococcus contains within itself two main antigenic principles which are quite distinct from one another :

1. The first of these is the bacterial protein which, when injected into an animal such as the rabbit or horse, evokes the production of agglutinins, opsonins, lysins, and complement-fixing antibodies. This protein is complex ; it contains primarily and in chief abundance the specific antigen of its particular type ; secondarily and in lesser degree antigenic elements of other types of the meningococcus and of other Gram-negative cocci, including at any rate in Types II and IV elements of the gonococcus.

2. The second antigenic principle contained by the meningococcus is totally distinct from the first, and is a powerful toxin. During life of the meningococcus this endotoxin is bound up with the bacterial protein with which it is apparently in some kind of union. When the coccus dies, or undergoes lysis, its intracellular toxin is liberated. The importance of this endotoxin lies in the fact that it is undoubtedly the chief cause of the pathogenic effect of the meningococcus. The endotoxin is remarkably stable ; cocci containing it can be heated for half an hour to 100° C. without impairing it.

That the endotoxin of the meningococcus is not a mere protein-split product as Vaughan and others would doubtless maintain is proved by the fact that with sufficient care and perseverance a specific antibody can be prepared that neutralizes it. In order to obtain antiendotoxin it is important to avoid overdosing the animal, and to test its serum at frequent intervals.

The endotoxin of the meningococcus is more specific even than the bacterial protein with which it is associated, since within the same type, as defined by agglutinin and agglutinin absorption tests, more than one endotoxin may be met with.

Therapeutic experience during the recent outbreak confirms these points. It is not sufficient for antimeningococcus serum to contain antibodies to the bacterial protein : the Type II serum agglutinated up to a high titre all of the strains of that type against which it was tested—nevertheless its therapeutic value was poor. On the other hand, the Type I serum prepared by Dr. Griffith and standardized by its antiendotoxic content was probably the most potent antimeningococcus serum yet used in this country, since out of 65 cases of what has been regarded clinically as the most severe form of cerebrospinal fever no less than 59 recovered when treated with this serum ; but its therapeutic value was limited to cases infected by Type I meningococcus. The reason why this serum failed in one of the few Type I cases that succumbed was investigated recently by drying the coccus isolated from this case and testing its endotoxin against the serum. In spite of the fact that the coccus was a typical specimen of Type I, and absorbed the specific agglutinin of that type perfectly, the endotoxin of this coccus was not neutralized by the serum, although this identical sample of serum was found to neutralize the endotoxin of other prevalent strains of Type I. For the rest, it is more than

likely that group antiendotoxins exist ; but if so their range is probably a limited one. The Type III cases that recovered showed this very clearly, since they required a Type III serum to cure them.

The types of meningococcus, therefore, are more complex than the study of agglutinin alone would indicate. Progress in the serotherapy of cerebrospinal fever would appear to require a systematic survey of the endotoxins contained within each of the four serological types of the meningococcus as defined by agglutinin ; and also better knowledge of the conditions under which antiendotoxin is formed. Neither investigation is easy ; but once such knowledge is obtained—and obtained it will be in course of time—it should be of the greatest value, for then it should be possible to prepare a therapeutic serum of standard quality as regards antiendotoxin content, capable of neutralizing the toxic principles of all the more common types of the meningococcus. With a serum such as this the clinical results already obtained with the Type I serum indicate that a very material reduction may be looked for in the at present unduly high mortality from cerebrospinal fever.

25. Bearing of the Preceding Observations on the Prevention, Diagnosis, and Specific Treatment of Cerebrospinal Fever.

It would appear that the meningococcus is a micro-organism of considerable complexity. Not only do antigenic tests prove that its protein belongs to one or other of four main types, but the intracellular toxin contained by this micro-organism would appear to be still more highly differentiated. In addition, it would seem that the meningococcus contains an exceedingly stable haemolytic substance, and a labile reductase, as well as the carbohydrate-splitting and proteolytic enzymes which have been studied by Vines.

The virulence of the meningococcus varies within wide limits, even when this capacity is tested as soon as possible after the coccus has been isolated from a severe case of cerebrospinal fever. The precise factors determining this virulence have still to be determined. For the present, all that can be said is that the virulence is, of course, entirely dependent upon the vitality of the coccus, and that it seems to be closely associated with the reducing power of the coccus on its immediate environment.

The observations recorded by Captain Glover prove that the best way to prevent cerebrospinal fever is to prevent overcrowding. It would seem that the strongest feature of the meningococcus is its capacity of maintaining itself in the nasopharynx of a chronic carrier for an extended period, and the weakest point of this micro-organism as a disease-producer is its rapid death outside the body. Hence the weakest point of cerebrospinal fever as an infective disease lies in the transit of the virus in air-borne droplets from nasopharynx to nasopharynx. How limited the range, and how dependent the successful transfer of infection is upon dosage is exemplified in the observations of Captain Glover, since a comparatively small increase in the distance between the men's beds was apparently sufficient to check the spread of infection. Some careful bacteriological observations recently published by Eagleton confirm this view. A further factor

that tends to keep down the incidence of cerebrospinal fever is undoubtedly the relatively high resistance which many people show to it—a resistance in remarkable contrast to that exhibited against influenza. There is reason to believe however that this resistance to the meningococcus is relative rather than absolute, and that it is certainly less in recruits than in older soldiers.

Experience and observation during the recent outbreak have taught us to take a broad view of this disease. It would seem that if bacteriology is to be applied in the most effective manner for the prevention of cerebrospinal fever, the bacteriologist should not wait until the disease manifests itself, but by swabbing samples of the population at intervals should keep a continual watch on the carrier rate of the community under his care—when the carrier rate rises, it is direct evidence—as Captain Glover shows—of overcrowding; when that is remedied, the carrier rate automatically falls. Cerebrospinal fever is thus a disease in which bacteriology is capable of rendering its most valuable service before the outbreak begins.

Where overcrowding is unavoidable, it is more than ever desirable for the carrier rate to be under observation. The results with the steam spray, though disappointing from the point of view of clearing up chronic carriers, are distinctly encouraging as regards checking the incidence of the disease. The secret of success would appear to be to submit not only contacts, but the whole community to the treatment. When some more efficient method has been elaborated for rendering the nasopharyngeal mucous membrane of a whole community unfavourable to the growth of the meningococcus still better results may be looked for.

The diagnosis of cerebrospinal fever is usually effected by lumbar puncture and examination of the cerebrospinal fluid. It should be borne in mind, however, that a meningococcus of the same type as that present in the cerebrospinal fluid of the patient is to be found in his nasopharynx. By swabbing the nasopharynx at the time that the lumbar puncture is performed much time may be saved in identifying the type of the infecting meningococcus. It may be mentioned that the area in the nasopharynx where the meningococcus occurs has recently been defined most carefully by Major Embleton and Dr. Bryant.

With regard to the specific treatment of cerebrospinal fever, experience during the recent outbreak indicates that considerable progress is to be made by further study of antiendotoxin. Although cerebrospinal fever is one of the few kinds of meningitis from which recovery may occur naturally, the high mortality from this disease when untreated shows that the treatment of cerebrospinal fever is a very severe test of the efficiency of any therapeutic serum. The results obtained with the Type I serum, therefore, are more than encouraging. They would seem to indicate that a future of considerable promise lies before other antiendotoxic sera not only in cases of cerebrospinal fever infected by other types of meningococcus, but possibly also in other still commoner infections, provided that the preparation of such antiendotoxic sera is pursued with sufficient diligence and resolution. At present antiendotoxin is probably the most difficult of all antibodies to prepare, and the conditions under which it is generated have still to be adequately defined.

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VI. The Laboratory Diagnosis of Acute Intestinal Infections, including the Principles & Practice of the Agglutination Test

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THE LABORATORY DIAGNOSIS OF ACUTE INTESTINAL INFECTIONS, INCLUDING THE PRINCIPLES AND PRACTICE OF THE AGGLUTINATION TEST

CONTENTS

PART I

THE LABORATORY DIAGNOSIS OF ACUTE INTESTINAL INFECTIONS

PAGE

I. Introduction 7

(1) General Scope of the Report 7

(2) The Bacterial flora of the Normal Intestine 8

(a) *The Gram-negative Bacilli of the Normal Intestine* 8

(b) *Streptococci* 13

(c) *Anaerobic Bacilli* 14

(d) *Scantier or more occasional Denizens of the Normal Intestine* . . 15

(e) *The Relation of the Normal Flora of the Intestine to Disease* . . 15

(3) The Bacteria associated with Specific Intestinal Affections . . 18

II. The Collection of Material for Laboratory Diagnosis . . . 19

(1) Faeces 19

(a) *Collection* 19

(b) *The Container and Mode of Transmission* 20

(c) *Transmission by Post* 22

(2) Urine 22

(a) *Collection* 22

(b) *Transmission* 23

(3) Blood 23

(a) *Amount of Blood necessary for making Cultures* 23

(b) *Methods employed for obtaining the Blood* 23

(c) *General Considerations governing Blood Culture* 28

(d) *Transmission* 31

III. General Considerations regarding Methods for the Isolation of the Pathogenic Bacilli 32

(1) Differential Media 32

(2) Enrichment Media 34

(3) Cultures from Faeces 36

(4) Cultures from Urine 40

(5) Cultures from Blood 40

(6) Cultures from other Secretions and Pathological Products . . 41

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PAGE

IV. The Specific Diagnosis of the Various Bacterial Infections . . 41

(1) The Enteric Group 41

(a) *Morphological Characters and Staining* 41

(b) *Biochemical Reactions* 42

(c) *Serological Tests* 44

(2) Bacillary Dysentery 46

(a) *Technique employed in making Cultures from the Faeces, &c.* . . 46

(b) *Identification of the Various Members of the Group of Dysentery Bacilli* 49

(c) *Serological Races of Flexner's Bacillus* 51

(d) *Schmitz' Bacillus* 53

(e) *Gettings' Bacillus* 53

(f) *Lactose Fermenting Types* 54

(3) Acute Food Poisoning 55

(4) Other Bacteria causing or associated with Intestinal Diseases . 57

(a) *Morgan's Bacillus* 57

(b) *B. pyocyaneus* 58

(c) *B. proteus* 59

(5) Cholera 60

(a) *Principles governing the Isolation of the V. cholerae* . . . 61

(b) *Methods employed in isolating V. cholerae* 62

(i) *From the Faeces of an Acute Case of the Disease* . . . 63

(ii) *From the Faeces of a Carrier* 63

(c) *Identification of V. cholerae* 64

(d) *The Question of Serological Types of V. cholerae* 66

(e) *Para-cholera and other related Vibrios* 66

V. The Diagnosis of Dysentery due to Animal Parasites . . . 69

(1) Amoebic Dysentery 69

(a) *Collection of Material, &c.* 69

(b) *General Remarks on the Preparation of Microscopical Specimens* . 70

(c) *Description of the Adult Form of E. histolytica and its Differential Diagnosis from other Amoebae, &c.* 71

(d) *The Identification of Precystic and Cystic Stages of E. histolytica* . 73

(e) *Differential Diagnosis of Precystic and Cystic Stages of E. histolytica from other Amoebae, &c.* 74

(f) *The Preparation of Stained and Permanent Specimens* . . . 77

(g) *E. histolytica in Preparations stained by the Method described, and its Differential Diagnosis* 78

(h) *The Diagnosis of Amoebic Abscess of the Liver or Brain* . . . 80

(i) *The Value of Positive and Negative Examinations for the Presence of E. histolytica in the Faeces* 81

(2) Balantidial Dysentery 82

Appendices 83

(1) The Carbinol Test 83

(2) Tests for Indol 83

(3) Brilliant Green Fluid Media 84

(4) The Preparation of solid Media for the Isolation of the Enteric Group and Dysentery Bacilli 85

(a) *MacConkey's Medium* 85

(b) *von Drigalski and Conradi's Medium* 86

PAGE

(c) *Endo's Medium* 86
(d) *Meyer and Stickel's Modification of Holt-Harris and Teague's Medium* 87

(5) Fluid Media used for the Isolation of *V. cholerae* 88
(a) *Peptone Water (Dunbar)* 88
(b) *Goldberger's Alkaline Egg Broth* 89
(6) Solid Media used for the Isolation of *V. cholerae* 89
(a) *Dieudonné's Medium* 89
(b) *Goldberger's Alkaline Egg Agar modified by Douglas* . . . 89
(7) The Haemolytic Test for the Differentiation of *V. cholerae* from Allied Vibrios 90
(8) Sugars in their Relation to Fermentation Reactions . . . 90

PART II

THE PRINCIPLES AND PRACTICE OF THE AGGLUTINATION TEST, WITH SPECIAL REFERENCE TO THE GROUP OF INTESTINAL BACILLI

I. Introduction 97
(1) The Phenomena of Agglutination 97
(2) The Nature of Agglutination 100
(3) The Physical Conditions influencing Agglutination . . . 101
(a) *Temperature* 101
(b) *Gentle Motion* 101
(c) *Nature of the Bacterial Emulsion* 101
(4) Other Factors influencing Agglutination 102
(a) *Spontaneous Agglutination* 102
(b) *Inhibition Zones* 102
(c) *Variations in Agglutinability* 102
(d) *Group Agglutinins* 103
(e) *Natural Agglutinins* 104
(f) *Multiple Agglutinins* 105

II. The Technique of Agglutination 106
(1) The Microscopic Method 106
(2) Intermediate Methods 108
(a) *Macroscopic Agglutination on a Slide* 108
(b) *Garrow's Agglutinometer* 109
(c) *Wright's Pipette Method* 110
(d) *Max Neisser's Method* 111
(3) The Macroscopic Method 111
(a) *The Bacterial Suspension* 112
(b) *The Preparation of Serum Dilutions* 114
(i) *Measurement by Pipette* 114
(ii) *The Drop Method* 118
(iii) *The Preparation of Serum Dilutions by the Drop Method* . . 122
(c) *The Dimensions of the Tubes* 124
(d) *Temperature and Time of Incubation* 124

PAGE

(e) *The End-point of the Reaction* 125
(f) *Illumination* 127
(4) Two Forms of Technique for the Macroscopic Method . . . 127
(a) *The Ordinary Method* 127
(b) *Dreyer's Method* 128

III. The Absorption of Agglutinins 137
(1) The Technique of the Absorption Test 137
(a) *The Dilution of the Serum* 137
(b) *The Bacterial Emulsion* 138
(c) *The Absorbing Dose* 138
(d) *Temperature and Duration of Incubation* 139
(e) *Sedimentation* 139
(f) *Provision of a Control* 139
(2) Description of an Absorption Test 140
(3) Multiple Simultaneous Absorption 141
(4) Reciprocal Absorption 143

IV. The Preparation of Agglutinating Sera 143
(1) The Route of Immunization 144
(2) The Form of Antigen 144
(3) The Immunizing Dose 145
(4) The Technique of Bleeding 146

V. Acid Agglutination 149
Appendix. Notes on the History of the Standardization of Agglutinable Bacterial Emulsions, by A. D. Gardner 152

Part I. THE LABORATORY DIAGNOSIS OF ACUTE INTESTINAL INFECTIONS

I. Introduction.

(1) General Scope of the Report.

The exact diagnosis of the enteric group of diseases, of dysentery, cholera, and other forms of enteritis rests largely on laboratory observations and the number and variety of the methods which may be employed are great. In approaching the subject of the standardization of these methods the Committee have been guided chiefly by considerations of practical utility. Simplicity, accuracy, and reliability are the chief requirements for a method in routine laboratory use. Methods which are so difficult and complicated as to be beyond the capacities of the ordinary worker, and which are suited only for specialized branches of research, will therefore be omitted or passed over with brief mention. It is, however, desirable in the case of simpler methods to explain clearly the principles upon which they are based, and to set forth in detail the exact technique by which they are best carried out.

The Committee fully recognize that the expert worker, who has been accustomed to obtain satisfactory results with his own methods, will be little inclined to change them. But improved methods are constantly being introduced and it is an advantage, even to the most practised worker, to review his procedure from time to time, and to adopt any new devices which are shown to possess advantages over the old. In this Report it is the aim of the Committee to sift the evidence for and against different methods of procedure, and to state their opinion as to which are the best adapted for ordinary laboratory use.

Some approach to uniformity of method is clearly desirable, if only in the interests of statistical research in which the experience of different workers has to be collated. But as in the case of their reports on standard methods in the diagnosis of Venereal Diseases, the Committee have no wish to urge the adoption by every worker of an absolutely rigid procedure, for this would be a bar to further improvements. It is, nevertheless, clear that certain methods are, for certain purposes, better than others, and it is this the Committee would emphasize, while leaving to the individual worker a reasonable latitude of choice. It is to be desired that every worker should be well aware of the limitations and fallacies that may be inherent in the particular methods he uses, and that, in recording his results, he should be careful to record also the full details of the technique employed.

(2) The Bacterial Flora of the Normal Intestine.

For reasons which will presently appear, it is desirable to preface any discussion of the bacteriology of the diseased intestine by a short account of the bacteria found there in health. All observers agree that the intestinal contents are sterile at birth, but that some of the characteristic faecal bacteria begin to appear in a few hours, even in the breast-fed infant, and thenceforward increase in number and variety. The faeces soon swarm with micro-organisms in numbers which have no parallel elsewhere about the body, an ordinary count showing hundreds of millions of bacteria per gramme. Stained films suggest that the numbers revealed by cultural methods fall far short of the truth, and it is probable that our knowledge of the total flora of the bowel is very imperfect.

Bacteria are not present in equal abundance in all parts of the intestine. In the duodenum and upper part of the jejunum they are relatively scanty or almost absent, but they increase in number as the ileo-caecal valve is approached. The prolonged sojourn of the intestinal contents in the caecum and ascending colon favours bacterial multiplication, and here the numbers present probably reach a maximum. Old and hard faeces in the sigmoid flexure and rectum show, according to most observers, a decrease in number, perhaps associated with the more acid reaction which there prevails. Amongst the factors which probably govern the content of faeces in living bacteria, one of the chief is the hydrogen-ion concentration of the intestinal contents. It is certain, too, that diet[1] has an influence, and also that geographical conditions are not without effect, though exact data on these subjects are scanty. Further, just as the flora of the naso-pharynx is known to vary from time to time, it is probable that similar changes may occur, unperceived, in the bowel; we may be sure that the processes by which enteric and dysentery bacilli pass from one person to another serve also to convey less harmful microbes.

So far as our present knowledge goes the vast majority of intestinal bacteria belong to three groups: (*a*) Gram-negative bacilli of the so-called 'coli-group', (*b*) streptococci, and (*c*) spore-bearing anaerobic bacilli. These predominant organisms must be considered first.

(a) *The Gram-negative Bacilli of the Normal Intestine.*

Inasmuch as the colon bacillus, first described by Escherich in 1885, has always been regarded as the prototype of these bacteria, they are commonly called the 'coli-group', but the term is loosely used. Some mean by it only those organisms which strictly correspond with Escherich's bacillus in morphology and general cultural characters: others extend the term to cover Friedländer's bacillus and even organisms liquefying gelatine, such as *B. cloacae*. Such a wide extension is on the whole a source of loose thinking and confusion, though it is not easy to draw the line in so variable a group of organisms.

The generic characters which are common to *B. coli* and its immediate congeners are as follows:—they are Gram-negative bacilli,

[1] J. C. Torrey, *J. Med. Research*, 1919, 39, 415.

forming no spores, weakly motile or at times non-motile, the flagella being few in number and peritrichous in arrangement: they do not liquefy gelatine and they ferment glucose, lactose, and many other substances, with the evolution of gas. As a rule they are not capsulated organisms, but the presence or absence of a capsule can hardly be deemed of specific value, for the motile flagellate forms may at times be replaced by non-motile capsulated ones, yielding a viscid slimy growth on culture media. The cultural appearances are then so unlike those of the ordinary forms that they would be regarded as unrelated, were there not ample evidence of the transformation of the one type into the other. Such capsulation is most marked and most habitual in the '*B. mucosus capsulatus*' group (Friedländer's bacillus, &c.), but it is said to occur in *B. coli* and has even been noticed in *B. paratyphosus B.*[1]

Classification of the B. coli Group. Many names have been applied by different writers to bacilli conforming to this generic type, especially in the early days of bacteriology, and some of these names are sanctioned by long usage. But the original descriptions were often imperfect, and it is not always easy to be sure that we are nowadays using the names in the same sense as the original observer, for the characters by which we to-day endeavour to distinguish bacteria are widely different from those at one time employed, and our views as to specificity and variation amongst bacteria have also undergone considerable modification.

Even in the light of present knowledge it is a matter of great difficulty to classify the members of the group into distinct species. In morphology and mode of growth on ordinary culture media they resemble one another so closely that it is impossible to draw any specific distinctions. The serological criteria, which in so many bacterial groups serve as a court of appeal, seem here to fail us, for almost each individual strain of *B. coli* is apt to show its own serological peculiarities, and grouping has so far proved unsatisfactory. In these circumstances reliance has come to be placed upon the chemical changes set up in culture media by these organisms, which happen to be very active in this direction. Such physiological characters may be used in two ways. The simpler of the two is to note the capacity of the different strains to ferment a given series of sugars and similar substances. The other is to study the precise mode of attack upon a sugar or a protein, by a determination of the end products. The first method would merely determine the fact of fermentation of a given sugar and would rely for differentiation upon the particular sugars attacked. The second method, not content with determining the mere fact of fermentation, would go on to analyse the gas produced, and determine the ratio between the CO_2 and H_2; it would compare the relative amounts of such products as acetic acid and alcohol and ascertain the presence of substances such as those of the methyl-carbinol group and indol. By these means it can be shown that different species vary in the fundamental nature of their attack upon a sugar or protein. Examinations of this sort can only be carried out by trained chemists, but for routine laboratory use certain simple tests

[1] Fletcher, W., *Lancet*, 1918, ii, 102.

can be made to reveal most of the facts required. The 'carbinol' test, for example, is of great utility in indicating the nature of the attack on glucose, while the indol test indicates the breaking down of the tryptophane molecule.

In any estimate of the value of simple 'sugar tests' certain facts must be kept in mind. Individual strains may undoubtedly vary from time to time in their capacity for fermenting a given sugar. A lactose-fermenting strain may temporarily lose its power to ferment lactose. Twort and others have shown that a bacillus may be trained to form acid from a sugar which was at first not attacked, and Penfold's work on 'mutations' indicates that such changes occur spontaneously. By extending the range of fermentable substances employed as tests, the number of apparently different bacterial types revealed may be increased almost indefinitely, and far beyond anything that can be held to indicate limits to legitimate species. For practical purposes it is necessary to restrict the number of test substances to half a dozen or so, and even then it may be open to doubt whether the differences revealed are of specific value, though they are sufficiently constant to be of signal service in the provisional identification of certain common types of coliform bacteria.

Although the problem had been previously approached by workers abroad, the pioneer work on such lines in this country was that of MacConkey[1] (1905). This observer collated the characters attributed to the bacilli of the *B. coli* group by previous writers, and, having collected such authentic examples of the named types as he could, proceeded to subject them to a wide range of sugar tests, to which he added some of the more fundamental tests to which allusion has been made (indol and carbinol tests). He further examined in the same way a large series of strains which he obtained from the faeces of man and animals and from milk, with the view of establishing some set of fermentation tests which might offer a serviceable classification. For the common organisms which ferment glucose and lactose, he suggested the following grouping, based on their behaviour with saccharose and dulcitol.

		Saccharose.	*Dulcitol.*
I.	*B. acidi lactici* group	–	–
II.	*B. coli communis* group	–	+
III.	*B. Friedländer* group	+	+
IV.	*B. lactis aërogenes* group	+	–

This grouping involves certain anomalies. Thus the common variety of *B. coli* which attacks saccharose—the so-called *B. coli communior*—would fall into a separate group from the classical organism of Escherich, of which it is in all probability a mere variety, while certain organisms would be referred to the Friedländer group, which have little in common with the *B. pneumoniae* of that author.

Nevertheless, MacConkey's work gave a remarkable impetus in this country to the study of the Gram-negative bacilli of the intestine, and large numbers of subsequent observers have taken

[1] *J. Hyg.* 1905, 5, 333.

it as their model. The fermentable substances which he suggested as tests are still those which prove of most value in classification, namely, lactose, saccharose, dulcitol, and perhaps adonitol. By such means certain predominant types can be recognized amongst these organisms, and they appear sufficiently constant to deserve specific names, at least for the present. But they tend to merge into one another by intermediate varieties of lesser frequency and may at times, under culture, gain or lose a given sugar reaction, so that their constancy is not absolute. But the question of the nature and degree of specificity amongst bacteria is too large a subject to be considered here, and in a dominant and variable genus such as that represented by *B. coli* and its allies the difficulties which attend the matter present themselves in a peculiarly acute form. The most that we can safely attempt in the present state of our knowledge is a subdivision of the genus into groups rather than into defined species.

For this purpose the most fundamental character which we can take is the nature of the attack upon proteins and carbohydrates. The indol test is of value here, and also the carbinol reaction first described by Voges and Proskauer.[1] For the technique see Appendix.

The carbinol reaction is associated with other chemical features in the process of fermentation. Theobald Smith[2] found it correlated with the $\frac{H_2}{CO_2}$ ratio in the gas produced: with *B. coli communis* this ratio was $\frac{2}{1}$, but with *B. cloacae* $\frac{1}{2}$. Similarly Harden[3] found marked differences in the end products of the fermentation: with *B. coli communis* the molecular ratio between the acetic acid and alcohol formed was 1 to 1 or thereabouts, with *B. lactis aërogenes* it was 3 to 1 or 5 to 1, and with *B. cloacae* about 13 to 1. Facts of this nature are more significant for biological classification than mere ability to produce acid from this or that sugar.

This opinion becomes strengthened when we come to apply the known facts to the classification of the Gram-negative bacilli of the normal intestine. Capt. A. L. Robinson, R.A.F., M.S., has recently analysed the data for 2,100 such strains published by various observers, largely from India, but also from the Mediterranean and from England. This work is as yet unpublished, but by Capt. Robinson's courtesy the Committee is allowed to make use of his data. In the following table, modified from that constructed by Robinson by omitting certain details and expressing the results as percentages, the distribution of these 2,100 strains is seen according to the sugar reactions of MacConkey in the vertical columns, and according to the indol and carbinol tests in the horizontal columns. It is plain that the characteristic feature of the coliform bacilli of normal faeces is that they are distinguished by a positive indol and a negative carbinol reaction. No less than 93·4 per cent. of the strains form indol, while only 6 or 7 per cent. give the carbinol reaction. No such striking differentiation is offered by the sugar tests, and it may fairly be concluded that in public health work, in

[1] *Ztschr. f. Hyg. u. Infektionskrankh.* 1898, 28, 30.
[2] *Centralbl. f. Bakteriol.* [&c.] 1895, 18, 1. [3] *J. Hyg.* 1905, 5, 488.

which these organisms are regarded as the chief index of faecal pollution of water, much greater significance must be attached to the indol and carbinol tests than to the sugar fermentations.

	Sacch. – Dulc. –	Sacch. – Dulc. +	Sacch. + Dulc. +	Sacch. + Dulc. –	Total.
Indol + Carbinol –	41·9 % of which 7·5 % are *B. acidi lactici.*	22·8 % of which 0·3 % are *B. coli communis.*	12·6 %	14·5 %	= 91·8 %
Indol + Carbinol +	–	–	0·8 %	0·6 %	= 1·4 %
Indol – Carbinol +	0·1 %	–	1·3 %	3·3 % 2·5 % being *B. lactis aërogenes* and 0·8 % *B. cloacae.*	= 4·7 %
Indol – Carbinol –	0·5 %	0·3 %	0·6 % of which 0·4 % are *B. Friedländer.*	0·2 %	= 1·6 %
Total.	42·5 %	23·1 %	15·3 %	18·6 %	= 99·5 %

Grouping of 2,100 *coliform bacilli* (lactose fermenters) from normal human stools.

Compiled by A. L. Robinson from the following data.

Archibald (Egypt)	20 strains	Castellani (Ceylon)	104 strains
Clemesha (India)	1206 „	Hirst (Ceylon)	250 „
Houston (England)	88 „	MacConkey (England)	178 „
Robinson (Malta)	150 „	Med. Res. Com. (Mediterranean) No. V. Dysent. Report.	104 „

Nevertheless sugar reactions have proved of such general utility for at least a rough orientation of the different races of coliform bacilli that the use of these tests is likely to continue and it may be of service to give in tabular form the reactions of certain of the more prominent types, names for which are in current use.

Name of Bacillus.	Substances Fermented.					
	Glucose.	Lactose.	Saccharose.	Dulcitol.	Mannitol.	Adonitol.
B. coli communis	A & G	A & G	–	A & G	A & G	–
B. lactis aërogenes	A & G	A & G	A & G	–	A & G	A & G or –
B. acidi lactici	A & G	A & G	–	–	A & G	A & G
B. coli communior	A & G	A & G	A & G	A & G	A & G	–
B. cloacae	A & G	A & G	A & G	–	A & G	–

A & G indicates that both acid and gas are produced; – that no change is noticed.

The lactose test is commonly regarded as a fundamental one, and although, for reasons already given, its value must not be deemed absolute, it possesses considerable significance because it so happens that nearly all the more pathogenic intestinal organisms do not ferment this sugar. Lactose has thus come to be the chief ingredient in the differential media used in the search for such organisms. There are, however, organisms otherwise closely allied to *B. coli* which fail to ferment lactose or ferment it late: the name 'paracolon bacilli' has been applied to such types and they will be discussed later, since they can hardly be regarded as frequent members of the normal flora of the intestine.

(b) *Streptococci.*

Next in point of abundance, and sometimes even outnumbering *B. coli*, come the streptococci of the alimentary tract. The difficulties which attend the classification of the Gram-negative bacilli are equally present here, and no system which is likely to prove final has yet been reached. It is generally admitted that a legitimate distinction may be drawn between the virulent type known as *Streptococcus pyogenes* and the common saprophytic forms of the alimentary canal. A reaction of considerable importance in this connexion is that established by Schottmüller. In blood agar plates the colonies of *S. pyogenes* and its varieties are surrounded by a clear halo of haemolysis, while those of the common salivary and faecal streptococci produce no haemolysis, but a change of the haemoglobin into a substance giving rise to a greenish tint. The distinction between haemolytic and non-haemolytic streptococci seems to be correlated with virulence.

In 1906 Andrewes and Horder put forward a tentative grouping of the streptococci based on the sugar reactions introduced by Gordon. Some of the names which they suggested for the commoner types have found a certain degree of acceptance—e.g. *S. salivarius* and *S. faecalis*, but it appears likely that, as with the *B. coli* group, sugar reactions cannot be taken as furnishing a wholly satisfactory grouping, though they are certainly of use for provisionally referring a streptococcus to its sub-group, if a few leading sugar tests are alone employed. Houston[1] examined 300 strains of streptococci from normal faeces, using the eight tests put forward by Gordon, and he found them to fall into no less than forty groups—a result which is clearly of no practical utility. Much of the laborious work which has been expended in this direction serves, in fact, merely to illustrate the extreme variability of the streptococci in their powers of sugar fermentation, rather than to establish any satisfactory basis of classification. There are, however, certain sugar tests which have proved useful; the chief substances to be recommended are lactose, saccharose, raffinose, and mannitol. No serious attempt has yet been made to work out the serological relations of the streptococci, but certain observations which have been made suggest this line as perhaps more likely than any other to furnish a scientific grouping.

[1] L.G.B. Report, 1903–4, 33, 472.

Turning to the streptococci of normal faeces, we find a conspicuous absence of haemolytic types: *Streptococcus pyogenes* appears habitually absent from the healthy intestine. The types which are most abundant are short-chained, non-haemolytic ones. which, when tested upon the mouse, are found devoid of virulence. A type commonly found is that which Andrewes and Horder termed *Streptococcus faecalis*, which ferments mannitol as well as lactose and saccharose, but not raffinose. It is further distinguished from other streptococci by the vigour of its growth on culture media and by its greater hardiness and resistance to desiccation: it has, moreover, the property of forming sulphuretted hydrogen in broth. It is apparently identical with the 'enterococcus' of French writers. It may perhaps rank as a distinct species, but though common in the faeces it is not necessarily the most abundant streptococcus. Other types are found of more delicate habit and offering a close resemblance to those so abundant in the buccal cavity, which have hence been termed *Streptococcus salivarius*. These are of feebler growth, often refusing to grow except at body temperature, and they tend to ferment raffinose and not mannitol. The variety of intestinal streptococci is so great that it seems useless to attempt any classification of them, beyond the above general statements, at least in the present state of our knowledge. It may be added that although streptococci grow well on v. Drigalski and Conradi's medium, many of the more weakly sorts will not grow on MacConkey's bile salt lactose medium.

(c) *Anaerobic Bacilli.*

The conditions in the intestine are anaerobic, and indeed the *B. coli* group and the streptococci are facultative anaerobes. Our knowledge of the stricter anaerobic bacilli is more imperfect than in the case of the two preceding groups, since anaerobic plate cultures are rarely made from the faeces. By appropriate means these organisms can be cultivated, and are found to belong to a number of different species. *B. welchii* (*B. perfringens*) and *B. sporogenes* are constantly found, and it is probable that, when further attention has been paid to the subject, most of the anaerobes which have attained notoriety in septic war wounds will be found present in the normal intestine. Tulloch's observations show that the tetanus bacillus may sometimes be found in human faeces, as has long been known in the case of the horse and other animals.

Amongst the anaerobes, though not belonging to the group of sporing organisms which are usually associated with that term, may also be mentioned certain bacilli found especially in the faeces of suckling infants. Two names have been applied to them, *B. bifidus* and *B. acidophilus* (see Torrey).[1] They are Gram-positive organisms, strictly anaerobic, and apparently related to the diphtheroid group. The name *B. bifidus* relates to the branching forms seen in cultures. According to Tissier the intestinal flora of a purely breast-fed infant shows an overwhelming preponderance of these bacteria.

[1] Torrey, *J. Bact.* 1917, 2, 434.

(d) *Scantier or more occasional Denizens of the Normal Intestine.*

While it is certain that an overwhelming majority of the bacteria of normal faeces belong to the three groups already mentioned, there are other organisms which have been met with sufficiently frequently to gain the reputation of being normal to the intestine. The flora of the intestine is not a fixed thing for any individual; it must vary from time to time in accordance with the changing conditions of diet. New bacterial species must frequently gain access to the intestine, and some of these may succeed in establishing themselves there for awhile without necessarily causing any disturbance in the health of the host. We are liable, in short, to become temporary carriers of species which are non-pathogenic, just as we know may occur with pathogenic species. Plenty of instances are on record of the transient presence of the *Vibrio cholerae* and the typhoid bacillus in the faeces of contacts who have never presented any symptoms of disease. Non-pathogenic species are usually not looked for, and their discovery is a matter of accident when they happen to obtrude themselves upon our notice.

Amongst the species thus found at times in apparently normal faeces, the following may be mentioned: *B. faecalis alkaligenes*, a motile organism differing from *B. coli* in its inability to ferment any sugar, and also, it is said, by the fact that its flagella are not peritrichous, but in terminal tufts; *B. pyocyaneus*, readily distinguished by its pigment formation; *Proteus vulgaris*, never missed when present on account of its inveterate habit of overgrowing all the other colonies in a plate culture. These three organisms are perhaps those which have the best claim to be regarded as 'normal' to the intestine, apart from the main groups above described, but it is certain that they are quite uncommonly met with in healthy conditions. Numerous other species have indeed been described by different observers, but there is no evidence that they are of any importance and it is not worth while to enumerate them here. (Friedländer's bacillus and *B. cloacae* are doubtless truly normal in the intestine, and have been mentioned in discussing the *B. coli* group.)

(e) *The Relation of the Normal Flora of the Intestine to Disease.*

It is certain that, in the healthy body, this rich and varied intestinal flora is a purely saprophytic one. Nevertheless, it will be noted that the majority, and perhaps the whole, of the common bacteria of the bowel are potential parasites, capable, under suitable conditions, of setting up acute disease. We have, therefore, to consider the reasons for this normal tolerance.

In the first place, the body possesses, or evolves, protective mechanisms, humoral or phagocytic, against these normal tenants of the gut. It is known that they frequently escape in small numbers into the lymph-stream and blood. It would be incredible that they should not, considering the facilities for absorption from the intestine, but we have direct evidence that they do so escape. Conradi[1] examined the viscera of freshly slaughtered healthy

[1] *München. med. Wchnschr.* 1909, **56**, 1318.

animals, using elaborate precautions against accidental contaminations. He was able to demonstrate, by incubation of blocks of tissue, sterilized on the surface, and subsequent bacteriological examination, that 66 per cent. of the livers, 30 per cent. of the muscles, 31 per cent. of the kidneys, and 9 per cent. of the spleens examined, yielded bacteria, and, in order of frequency, the three organisms most often found were *B. coli communis*, *B. lactis aërogenes*, and streptococci. This points clearly to an intestinal origin for these wanderers, and their special abundance in the liver suggests the portal system as the probable channel of ingress. Their life in the body outside the intestine is doubtless short, but the constant access to the blood and tissues will readily account for the development of antibodies against them.

Nevertheless, it would seem that this general defence is not of a very high order. The diminished resistance of the body in impending death is not rarely associated with a so-called 'agonal' or 'terminal' infection of the blood-stream with one or another of the common intestinal bacteria. Further, when such bacteria gain access to tissues not habituated to their presence they may readily set up acute disease. The effects of *B. coli* in the urinary tract, of intestinal streptococci upon the endocardium, and of *B. welchii* on the muscles bear abundant witness to this truth. We must therefore look to some local defence in the intestine itself to explain its complete immunity in health. A local tolerance of this sort is not confined to the intestine: it is seen equally in the mouth and in the skin, which also swarm with 'normal' microbes, though their flora differs from that of the gut. By some mechanism which has not yet received any explanation, the intestinal tissues manage to keep at bay the multitude of micro-organisms with which they are in contact, so long as these organisms are those to whose presence they are habituated.

There is evidence that in acute intestinal disease of any sort the normal bacteria of the gut are apt to acquire an enhanced virulence. Thus it is stated that if intestinal obstruction be artifically induced in an animal, the *B. coli* recoverable after death from the peritoneal cavity are of abnormal virulence. In acute diarrhoeas, and also in typhoid fever, *B. welchii* has been found much more abundantly than in health, and apparently in a more virulent condition. And not only may the pathogenic powers of the normal flora be exalted in intestinal affections, but its character may change. The relative proportions of its different members may be altered, or unusual types of bacteria may appear.

It follows that, in the search for a causal agent in diarrhoeal affections, extreme care must be exercised in interpreting the results of cultures from the stools. It would seem possible for an unusual bacterium to be present in such large numbers in the stools under these circumstances as to suggest its causal relation with the disease: yet the actual cause may be something quite different. We must not too hastily assume that because *B. pyocyaneus* or *B. proteus* is abundant in the stools of a case of enteritis it is necessarily the primary cause: it may be that the abnormal conditions set up in the intestine by some other cause have favoured the multiplication of

one of these organisms and so allowed it to exhibit a deceptive prominence.

Lack of caution in weighing such possibilities has probably led, in the past, to a good many loose inferences as to the causation of enteritis. Before we can justly accuse an organism of connexion with enteritis there must be some reasonable degree of conformity with the so-called Koch's postulates. The specific organism must be demonstrable in a considerable proportion of the cases in an outbreak, and not in an isolated case, and pure cultures must be shown capable of setting up an enteritis in susceptible animals or in man himself. It is perhaps too much to demand that specific antibodies must necessarily be found in the serum of convalescents, since in most cases the infection may well have been a local one without invasion of the blood-stream. Nevertheless, positive blood-cultures, or the demonstration of antibodies in the blood, would immensely strengthen the evidence. In the case of Gaertner's bacillus, and perhaps less convincingly in that of Morgan's bacillus, evidence of this kind is forthcoming, and we can accept these organisms as causes of enteritis, but with *B. proteus* and *B. pyocyaneus* we are on more doubtful ground.

There is, however, another side of the problem. It is within the range of possibility that certain forms of enteritis may be due to organisms which are normal inhabitants of the intestine, but foreign to the individual concerned. It has been asserted that individuals have their own personal strains of *B. coli*, and the facts of agglutination certainly indicate serological variations amongst strains from different persons. We probably interchange our intestinal bacteria pretty freely, but little harm results as a rule from this where the personal strains of a community have much in common. But if it is true that intestinal bacteria are kept at bay by a local immunity of the bowel against the races to which it is habituated, it is probably also true that this local immunity will fail if the bowel is invaded by a foreign set of bacteria. It is notorious that a new-comer to a tropical place often gets diarrhoea soon after his arrival, and is assured by the residents that this is the rule and that it will pass off as soon as he is acclimatized. One possible and even likely explanation of this would lie in the invasion of his intestine by a new set of bacterial races, to which in a short time he will in his turn become accustomed. When our troops landed in Gallipoli practically every man got diarrhoea in a few days, and solid stools were almost unknown. Aided by flies, they began to partake of the faecal bacteria of the Turks from the day of their arrival: some of these bacteria were of known pathogenic species, and dysentery and enteric fever were correspondingly rife, but much of the diarrhoea seems to have been of a non-specific nature and might well have been due to the cause above suggested. It is certain that when these cases of enteritis returned convalescent to this country, plate cultures from their faeces displayed a remarkable assembly of unfamiliar bacteria, including many lactose-negative forms, such as paracolon bacilli, and that on recovery over here this variegated flora gradually returned to that customary in this country.

Paracolon bacilli. The prefix 'para', in bacterial nomenclature,

usually suggests an incomplete stage in our knowledge. Types which are evidently allied to known species, but differ from them serologically or in other respects, can be easily, if loosely, labelled with the epithet, which may serve for the time being but requires the work of succeeding observers for confirmation or the reverse. Sometimes the prefix sticks, as in the case of the paratyphoid bacilli, where indeed it has this justification—that the disease produced, as well as the bacillus causing it, presents a marked similarity in the two cases. In other instances, as with the parameningococcus or *B. paramelitensis*, further knowledge proves that we are dealing merely with serological races of one and the same organism, and the prefix 'para' becomes devoid of significance. The term 'paracolon bacillus' falls into neither of these categories and has neither the justification of the former nor the excuse of the latter. If we continue to use it, it is only because no one has yet studied this group of organisms sufficiently to make it worth while to bestow proper scientific names upon its members.

Gilbert,[1] in 1895, used the name 'paracoli-bacilles' merely as an appellation for bacilli related to, but not identical with, *B. coli*, including Pasteur's 'bacille lactique', *B. lactis aërogenes*, and the bacillus which Gilbert and Lion had isolated from a case of ulcerative endocarditis. He placed in this group coliform bacilli which formed no indol or failed to ferment lactose, and he evidently had no intention of defining by the term any one species of bacillus.

Since that time various writers have employed the name in different senses, but usually loosely. As the various members of the *B. coli* group have become better defined and named, the term has become more restricted in its use and most observers now apply it to bacilli resembling *B. coli* but failing to ferment lactose, or fermenting it late. Such organisms form acid and gas in glucose, in mannitol, and often in saccharose and dulcitol also. There are evidently several varieties, but whether they form a species including different races or, as is more probable, a group containing several species must be decided by future observation. Herrold and Culver [2] have studied these bacilli serologically and find them distinct from *B. coli*: the paracolon strains studied, 43 in number, which were from the urine, appeared to fall into four serological races. Bacilli of this group are rarely met with in the faeces of healthy persons in this country, though they are met with in cases of intestinal disturbance, especially in the East. There is, however, little or no evidence of their specific causal relation to intestinal disease, common though they are as associated organisms.

(3) The Bacteria associated with Specific Intestinal Affections.

It has already been suggested that the access to the intestine of any foreign set of bacteria—bacteria, that is, to which the intestine of the host is not habituated—may give rise to transient enteritis and diarrhoea of a more or less non-specific character. There are, however, a number of bacterial species of higher pathogenic power, capable of more serious attack upon the intestinal mucosa or of

[1] *Semaine méd.* 1895, 25, 1. [2] *J. Infect. Dis.* 1919, 24, 114.

invasion of the blood-stream. It is with the recognition of these agents of specific disease that the following pages chiefly deal.

Even *B. coli* has been asserted to give rise to a septicaemia under certain circumstances, such as the grossly insanitary conditions in which some of our troops found themselves in Gallipoli and elsewhere in the East. Such *B. coli* septicaemias resembled mild enteric fever, and were rarely if ever fatal. The same is certainly true of *B. faecalis alkaligenes*. But for the most part the fevers with which we have to deal are due to well-defined bacteria which are not inhabitants of the normal intestine, but which spread from case to case in epidemic fashion, more especially under insanitary conditions. They will be most conveniently discussed under *clinical* headings.

The first group comprises the *enteric fevers*, typhoid and paratyphoid, in which there is a septicaemia as well as an intestinal infection. The second group is that of the *dysenteries*, in which the infection is almost invariably limited to the intestinal tract: bacillary and amoebic dysentery will be considered separately. The third group consists of one disease only—*Asiatic cholera*, likewise limited to the intestine. In a fourth are the *food-poisoning cases*, in which enteritis is due to organisms of the Salmonella group.[1] Lastly comes a less distinct fifth group, in which is placed Morgan's bacillus as a definite cause of enteritis and a probable agent in the production of *summer diarrhoea*. Here also may be included *B. proteus* and *B. pyocyaneus*.

The consideration of these groups will be preceded by a general account of the methods employed in the collection of material for bacteriological diagnosis, and followed by a section on the subject of agglutination methods.

II. The Collection of Material for Laboratory Diagnosis.

(1) Faeces.

(a) *Collection.*

There is a widespread ignorance of proper methods of collecting and forwarding excreta to the laboratory for diagnostic purposes. Whether in private or in hospital practice the medical man rarely himself obtains the required samples: he leaves that either to the nurse, or orderly, or to the patient. But this does not relieve him of responsibility. It is he who needs the services of the bacteriologist, and he should, therefore, instruct whoever collects the sample, and see that the instructions are carried out. Too often he appears to be wholly ignorant of his duties in this respect, with the result that material which is useless arrives at the laboratory.

The considerations determining proper practice are simple and obvious.

Normally the contents of the jejunum are almost sterile and it is not until the lower part of the ileum is reached that many living bacteria are found. These reach their maximum in the last few feet of the ileum and the commencement of the large intestine.

[1] This generic term has been introduced for *B. suipestifer*, *B. aertrycke*, the paratyphoid organisms, and Gaertner's *B. enteritidis*.

In the remainder of the large intestine, as the contents become more solid and their reaction tends to change from alkaline to acid the number of living bacteria decreases, and this is especially the case in respect of the coliform group of bacilli.

It is therefore clearly indicated that the more nearly the sample examined approaches in character to the contents of the intestine as they reach the caecum, the more likely are satisfactory results to be obtained, and experiments carried out on man have demonstrated conclusively that this is the case. The looser the stool the better.

Loose stools in fact contain enormous numbers of living bacteria; it is therefore unnecessary to forward faeces to the laboratory in bulk or to transmit the entire motion. *One cubic centimetre of a loose motion, properly selected and transmitted, is ample for all bacteriological purposes.*

Such loose motions are common in acute intestinal disease, whether of the ileum (cholera and the typhoid group) or of the colon (bacillary and amoebic dysentery). In the case of carriers or suspected carriers, particularly when there is any tendency to constipation, if a sample of faeces is desired for bacteriological purposes, a purgative should be given and some of the resulting loose stool be forwarded. The examination of a constipated motion in the case of a carrier is a worthless procedure.

To this general rule there is one exception, namely, in cases of known or suspected chronic amoebic dysentery where search is being made for encysted forms of *Entamoeba histolytica*, the solid faeces should be examined. If the loose stools only of carriers be examined the small vegetative stages of the amoebae are found, and these are difficult, if not impossible, to diagnose.

In cases of Asiatic cholera it is in the epithelial flakes of the rice-water stools that the vibrios are present in greatest abundance. A sufficiency of the rice-water stools must be sent to the laboratory to ensure that some of these flakes are present.

(b) *The container and mode of transmission.*

The container must be watertight, and capable of being sterilized. A small wide-mouthed bottle, which with its cork is sterilizable in the autoclave, fulfils all these conditions, as will also a small, corked test-tube of stout glass. The common sterilized 'swab' in its test-tube can even be employed and the wire used to collect the material. The swab, however, runs the risk of becoming dried up: a few drops of boiled water at the bottom of the tube prevent this. Swabs are more suitable in hospital practice than for transmission to a distance.

For general purposes and transmission by post the best container so far devised is that indicated in Fig. 1, namely, a small wide-mouthed bottle, about 7×3 cm. provided with a cork. Inserted into the cork is a small spoon of white metal, having a bowl large enough to contain 1 grm. or a little more of faecal matter. The spoon is used to collect the material and holds it, if semi-solid. If the motions be liquid two or three spoonfuls are

transferred to the bottle. The whole apparatus is sterilized in the autoclave before use.

The bottle should be clearly labelled with the patient's name, the date, and the hour of evacuation, and with the nature of the examination requested.



Fig. 1.

The sooner cultures are made after passage of a stool the greater the likelihood of obtaining positive results. There should, therefore, be no delay in making cultures when the specimen has been received at the laboratory.

Micro-organisms, it is true, vary in their resisting powers. Typhoid bacilli have been isolated from faecal matter after several days, but often, especially in fluid stools, if kept, they are rapidly overgrown by saprophytic forms. According to Friedemann[1], dysentery bacilli are much less viable, and may actually disappear from the stools within one hour after passage. Most observers agree that they are not as a rule recoverable after six to eight hours. The collection of material direct from lesions by means of the rectal speculum has been suggested.

A method of preserving faeces for delayed examination, introduced by Teague and Clurman,[2] consists in emulsifying the faeces, obtained as soon as possible after evacuation, with about double the volume of 30 per cent. glycerine in 0·6 per cent. NaCl solution. It has been found that this procedure is of value in preventing typhoid and paratyphoid bacilli, if present in the original specimen of faeces, from being overgrown by the ordinary coliform organisms. This method should be employed whenever bacteriological examination of faeces is to be delayed, e.g. when the specimen has to be transmitted for a distance.

This method, as has been shown by both Ellis and Fletcher, is also applicable to the preservation of dysentery bacilli in faeces. In all cases care should be taken that the glycerine employed is of good quality and free from acidity.

Rectal swabs.—Instead of examining a portion of a stool, equally good results may, especially in cases of dysentery, be obtained by making the cultures from a swab taken from the rectal mucous membrane. This method is convenient in that the material can be secured independently of the passage of a stool. All that is required is a stout wire, some 8 inches in length, carrying at one end a firmly affixed swab of cotton wool. The swab is carried in a test-tube, and sterilized in the usual manner. In obtaining the

[1] *Deutsche med. Wchnschr.*, 1917, 43, 1524.

[2] Teague and Clurman, *J. Infect. Dis.*, 1916, 18, 653. Benians, *Lancet*, 1918, i, 255.

specimen the swab can be passed into the rectum alongside the finger, or preferably, through a narrow glass speculum, with a rotary movement in the same direction as the swab is rolled. It should be introduced as high as possible and then withdrawn with a similar rotary movement. These manipulations are said to cause no discomfort to the patient. A few drops of sterile water at the bottom of the tube prevent desiccation, but cultures should be made as quickly as possible—at the latest within four hours. This method has been extensively used in France during the late war in cases of dysentery, and the percentage of positive results was quite equal to that obtained by the examination of the stools.

(c) *Transmission of samples by post.*

Attention is drawn to the regulations governing the transmission by post of pathological specimens (abstracted from the official Post Office Guide):

'Deleterious liquids or substances, though otherwise prohibited from transmission by post, may be sent for medical examination or analysis to a recognized Medical Laboratory or Institute, whether or not belonging to a public health authority, or to a qualified Medical Practitioner or Veterinary Surgeon within the United Kingdom by *Letter Post, and on no account by Parcel Post*, under the following conditions:—

'Any such liquid or substance must be enclosed in a receptacle hermetically sealed, or otherwise securely closed, which receptacle must itself be placed in a strong wooden, leather, or metal case in such a way that it cannot shift about, and with a sufficient quantity of some absorbent material (such as sawdust or cotton wool) so packed about the receptacle as absolutely to prevent any possible leakage from the package in the event of damage to the receptacle. The packet so made up must be conspicuously marked "Fragile with care", and bear the words "Pathological Specimen".

'Any packet of this kind found in the parcel post, or found in the letter post not packed and marked as directed, will be at once stopped and destroyed with all its wrappings and enclosures. Further, any person who sends by post a deleterious liquid or substances for medical examination or analysis otherwise than as provided by these regulations is liable to prosecution.

'If receptacles are supplied by a Laboratory or Institute, they should be submitted to the Secretary, General Post Office, in order to ascertain whether they are regarded as complying with the regulations.'

(2) Urine.

(a) *Collection.*

Never forward to the laboratory the mixed urines of twenty-four hours, collected from a bedroom or sick-room receptacle. Such urine is already heavily contaminated with extraneous bacteria.

Never forward the first urine passed: this is always contaminated with bacteria from the urethra, meatus, &c.

The urine should when possible be collected through a sterile catheter as this is the best safeguard against contamination. Even in this case the catheter may introduce into the bladder some of the bacteria always present about the meatus, therefore the first

urine passed should be discarded. Whether a catheter be used or no, it is the middle stream that should be collected into a sterilized bottle.

Where a urine has not been collected by means of a catheter, cultures should be made immediately, as most bacteria multiply rapidly in urine. In the male the prepuce should be drawn back: in the female instruction should be given to separate the labia after cleansing the parts, thereby in either case reducing the contamination with extraneous bacteria to a minimum.

(b) *Transmission.*

The directions for transmission to the laboratory are the same as in the case of samples of faeces.

For bacteriological purposes (as distinct from chemical) a two-ounce bottle filled with urine is ample. The bottle should be stout, sterilizable, and preferably stoppered, or failing that, fitted with a good cork.

(3) Blood.

Diseases due to the enteric group of bacilli can undoubtedly be diagnosed more certainly and earlier in their course by means of blood culture than by any other method, and if this simple procedure were adopted the cause of many obscure fevers might more frequently be determined.

It is during the earliest stages of such diseases that the largest percentage of positive results is obtained; for instance, in cases of enteric fever 100 per cent. positive blood cultures have been obtained by several observers in uninoculated persons when the cultures were made during the first seven days of the illness. When the blood cultures are made during the second week the percentage of positive results is considerably smaller. Positive blood cultures have even been obtained during the incubation period, that is to say, before any symptoms of the illness have made their appearance. Blood cultures should therefore be made in all cases of fever in which the temperature remains elevated for more than two or three days, otherwise, especially in mild illnesses, the causal micro-organism may remain undetected.

(a) *Amount of blood necessary for making cultures.*

The quantity of blood required to make cultures is about 10 cubic centimetres. This quantity is sufficient for the inoculation of media of several kinds, and for duplicating the cultures so that some can be incubated under anaerobic conditions.

(b) *Methods employed for obtaining the blood.*

This quantity of blood is most easily obtained by drawing off a syringeful from one of the veins situated at the bend of the elbow.

The technique of this procedure will now be given, and afterwards alternative methods will be described.

Syringe.—The best type of syringe is either one of the 'Record' pattern which has a metal plunger and end-pieces while the barrel is of glass, or an 'all-glass' syringe.

Method of testing the syringe.—Before using the syringe it should be tested to ascertain that the plunger fits accurately. This should be done by drawing out the piston to its full extent, closing the end of the nozzle with one finger and then pressing home the piston ; if the plunger does not fit accurately air will pass between it and the barrel as soon as the pressure is raised, and, therefore, on releasing the pressure, the piston will not be forced upwards by the expansion of compressed air, as happens when the piston fits accurately and no air has escaped.

The alternative method, in which the nozzle is first closed and the piston then withdrawn, should never be employed, as in an accurately made syringe the piston on being released will fly back with such force that the barrel may be broken.

Sterilization of the syringe and needle.—The sterilization of the syringe is best carried out by boiling. This should be done as follows : the syringe is dismantled, the plunger being taken out of the barrel and the needle from the nozzle; the parts are then placed in warm water in any suitable vessel and the temperature raised until the water is boiling briskly ; it should be kept in the boiling water for 5 to 10 minutes. The syringe must be allowed to cool before it is again assembled, as not only is it very difficult to handle when hot but in the case of the 'Record' type, the piston being of metal expands so markedly with the increased temperature that it cannot be returned to the barrel until it has cooled down.

Care should be taken to fit the needle firmly on to the nozzle ; this should be done with a pair of strong sterile forceps and the needle and needle end of the syringe should at once be wrapped in a sterile cloth or placed inside a sterile receptacle of glass or metal, care being taken that the point of the needle is not injured.

The needle should not be too small or too short ; its length should be at least 3 centimetres and the external diameter from 0·75 to 1 mm.

It is very important that the needle point should be sharp and the surface quite smooth. Prolonged boiling in water tends to blunt the point and roughen the surface. To obviate this needles may conveniently be sterilized by heating them in olive, or any salad oil, until the oil smokes—a temperature of at least 150° C. Needles are best stored in oil that has been heated to remove any traces of water.

A method of ascertaining if the needle point is perfect is to pass it through a piece of tightly stretched thin leather by a quick prick ; if the needle is really sharp and smooth it passes through the leather without making any sound ; if, however, the point is not perfect a distinct noise can be heard.

Selection of vein.—The vein from which the blood may best be taken is usually the median basilic. Other veins at the bend of the elbow may appear more prominent and larger, but, owing to the ease with which they move about in the subcutaneous tissue, it is much more difficult to be certain of making the needle point enter their lumen.

Disinfection of the skin.—In most cases of patients in hospitals or in private practice in this country no complicated procedures are necessary. The skin at the bend of the elbow should be washed

with soap and water, dried with a sterile towel and then wiped over with a sterile swab soaked in some antiseptic solution, such as alcohol followed by ether.

Iodine is unsuitable, as it stains the skin, and has been shown experimentally not to be very efficient.

Where the skin is very dirty, as may be the case in soldiers on active service, much greater care should be exercised. The best method is to wash the skin thoroughly over the bend of the elbow with hot soap and water, and then to paint the area with a solution made by dissolving 0·5 per cent. each of brilliant green and crystal violet in 50 per cent. alcohol, as recommended by M^{c}Leod and Bevan-Brown.[1] The area should then be covered with dry, sterile gauze, which is kept in place for 10 to 15 minutes. This solution renders the skin quite sterile even after it has been fouled with highly resisting spores.

The method of drawing off the blood.—If the patient is in bed, the arm, resting securely on a pillow, should be fully extended with the bend of the elbow uppermost; at the same time it should be partially abducted from the body. A bandage is then applied round the middle of the upper arm firmly enough to arrest the flow of blood through the veins, but not so tightly as to compress the arteries; in consequence the limb below the bandage rapidly becomes engorged with blood and the veins at the bend of the elbow usually stand out distinctly. In place of a bandage the inflatable collar which is fitted to blood-pressure apparatus can be conveniently employed, as by its use the pressure can be very accurately gauged and can be at once reduced by opening the valve provided for that purpose.

The syringe is taken in the right hand, the skin being rendered tense with the left, and the needle pushed sharply through the skin, the barrel of the syringe being held almost parallel to the long axis of the arm so that the needle passes obliquely through the skin, subcutaneous tissue and, finally, the wall of the vein. The syringe is then transferred to the left hand and kept quite still; the piston is slowly withdrawn and, if the needle point lies in the lumen of the vein, blood will be seen to flow into the barrel.

It is quite immaterial whether the needle enters the vein in the direction of the blood flow or against it; usually it is more convenient to make the puncture towards the shoulder rather than towards the hand.

If on withdrawing the piston no blood appears in the barrel the needle point has not reached the lumen of the vein, and most probably is lying superficial to it; the syringe should therefore be raised slightly away from the arm and the needle pushed a little deeper, and on again withdrawing the piston the blood should now flow into the barrel.

It is important that when the blood is being drawn into the syringe the needle point should be kept perfectly still, for if movements are allowed to take place not only may the point slip out of the vein but the wall of the vein may be injured.

[1] M^{c}Leod and Bevan-Brown, *J. Path. and Bacteriol.*, 1918, 22, 74.

As a rule no difficulty arises, but in some cases, especially in fat persons and young children, or in subjects who, from some cause such as shock, or other conditions where the veins are underfilled, it may be difficult to find the vein.

In such cases it should be remembered that it is frequently possible to feel a vein, as a tense elastic band, even when it is invisible. It is also possible to make the veins more than usually prominent by keeping the hand and forearm in a dependent position in a basin of hot water, or better still, by wrapping the arm in a very hot fomentation. In all these procedures aseptic precautions must be observed.

Very occasionally in nervous subjects the veins show a tendency to contract, especially if the lumen is not found at the first attempt; if hot fomentations, &c., fail to remove this spasm the inhalation of a capsule of amyl nitrite will at once cause the veins to dilate to their full extent.

If after two or three attempts the lumen of the vein cannot be found it is advisable to loosen the bandage, withdraw the needle, and to begin afresh on the other arm.

As soon as the syringe has been filled with blood the bandage or inflatable collar should be loosened and the needle quickly withdrawn from the vein and pressure made with a sterile swab over the puncture. If this is done quickly no haematoma will be formed and the puncture in the skin will be scarcely visible. If, on the other hand, the needle is withdrawn before the bandage is loosened, a haematoma will invariably form which will lead to a considerable discoloration of the skin persisting for several days.

In general no dressing need be applied to the puncture.

Having obtained the requisite quantity of blood two courses are now open: (1) the various cultures are made at once, (2) the blood is transferred to some convenient vessel with the addition of one or other of the substances to be mentioned later and dispatched by post or messenger to the laboratory.

Whatever course is pursued the syringe should be emptied without delay and should be thoroughly washed out and subsequently boiled.

Before the procedure of making the cultures is detailed, or the treatment of the blood before it is sent to the laboratory is described, the methods which may be used for procuring the blood other than by means of a syringe will be dealt with.

Alternative methods of drawing off the blood.—If no syringe is available it is by no means difficult to insert a medium-sized needle in the median basilic vein and to collect the blood as it flows out of the end. When a short needle is employed it is advisable to attach a length of rubber tubing. In using this apparatus it is of importance to keep the pressure of blood in the veins as high as possible, and it is therefore necessary to adjust with care the pressure of the bandage which controls the flow of blood through the veins.

The operation is carried out in the same way as when a syringe is used, the needle being held almost parallel to the arm and pushed

obliquely through the skin and subcutaneous tissue into the lumen of the vein. When this is reached the blood begins to drip from the free end and is collected directly into a sterile test tube.

The Keidel tube.—Another ingenious apparatus has been devised which at the same time makes a syringe unnecessary and provides the vessel in which the blood is collected and sent to the laboratory. This is the so-called Keidel's apparatus, Fig. 2, which consists of a test-tube drawn out at the open end to a fine point. The tube is then sterilized and the narrowed portion is drawn out to a capillary tube. A small quantity of sterile water (2 or 3 drops is quite sufficient) should have been introduced before sterilizing. The capillary portion is now broken off very near the thicker portion and the contained few drops of fluid are boiled by passing the tube,

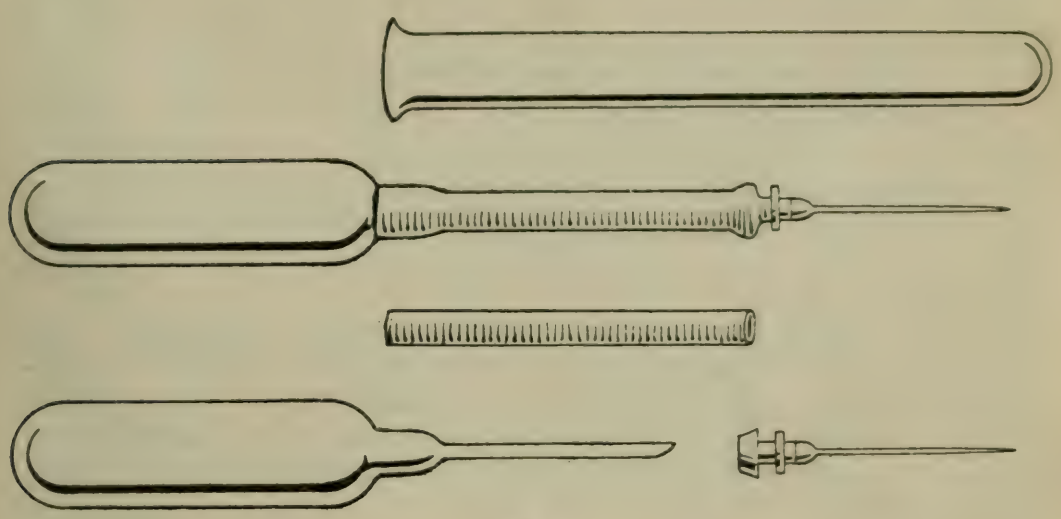


Fig. 2.

held in forceps, through the flame of a Bunsen burner. When the water has almost boiled away the tube is held narrow portion downwards to expel the last drops of fluid and the capillary end is at the same moment sealed in the flame. The boiling of the few drops of water makes sufficient steam to expel all the air, so that the tube contains nothing except water vapour. A rubber tube is then fitted over the drawn out end, and to the distal end of this a suitable needle is attached. The needle and rubber tube are sterilized by boiling. For carriage the tube and needle may be protected by a sterilized test-tube as shown in the illustration.

To obtain the blood the needle is passed into the lumen of the vein in the same way as when a syringe is used; this having been accomplished the tip of the narrow drawn out portion of the tube, which lies inside the rubber tubing, is broken off by crushing it with a pair of forceps or with the fingers. As the tube only contains water vapour the blood is sucked into it with considerable force until practically the whole apparatus is filled.

The disadvantage of this apparatus is that if the needle point does not lie in the lumen of the vein when the point of the tube is broken air may be sucked in through any ill-fitting joint while the necessary manipulations are being made to find the lumen of the

vein; in these circumstances the only course is to fit another exhausted tube to the rubber.

Wright's capsule.—If no needle or such apparatus as a Keidel's tube is available sufficient blood may as a last resource be collected, either from the ear or the finger, into a large Wright's capsule or a series of capsules. In such cases the skin over the area from which the blood is taken should be carefully sterilized, and a puncture rather larger than usual should be made so that a free flow of blood may be assured.

Splenic puncture.—The method of obtaining blood by splenic puncture is not to be recommended in the diagnosis of this group of diseases on account of the danger of subsequent internal haemorrhage.

(c) *General considerations governing blood culture.*

Before proceeding to describe the manner in which the various cultures are made or the best methods of sending the sample of blood to the laboratory, it will be necessary to discuss certain theoretical points which are of great importance, and without the knowledge of which it is difficult to follow the *raison d'être* of certain methods.

It has long been recognized that freshly drawn blood has very marked anti-bacterial properties, in consequence of which micro-organisms have great difficulty in proliferating. Amongst the factors militating against bacterial growth may be mentioned the following, the one of greatest practical importance being probably coagulation:

(i) The bactericidal power of the serum or plasma.

(ii) Inhibitory power. Blood plasma and blood serum have a very considerable power of inhibiting the growth of bacteria, although they may be unable to kill them.

(iii) Phagocytic power of the blood. Recent researches made by Wright and his fellow-workers have definitely shown that even after clotting has taken place the leucocytes have the property of freely moving about and ingesting any bacteria that may be present. They have also shown that a large number of the bacteria taken up by the leucocytes are killed.

(iv) Coagulation. The clotting of the blood has been shown to have a mechanical effect which tends to limit the growth of any bacteria that may be present. Thus Jousset showed that even if blood be diluted tenfold with distilled water and allowed to clot, all the bacteria that may be present in the particular sample are entangled in the fibrin meshwork that is formed, leaving the resulting serum germ-free.

In the past it has been frequently noted that positive blood cultures only show growth after a considerable period of incubation. To obtain a larger percentage of positive results and to be able

to give a positive diagnosis as rapidly as possible numerous improvements in the technique of making blood cultures have from time to time been introduced. On examining these it is at once apparent that all of them tend to neutralize, or diminish, the above factors.

Amongst other methods the following may be mentioned:

(i) That of greatly diluting the blood with ordinary broth. By using this method it has been shown that in the case of patients in the early stages of enteric fever the disease can be diagnosed in 100 per cent. of the cases when not more than 0·5 c.c. to 1 c.c. of blood is added to 500 c.c. of broth, while if the blood is added in the proportion of 1 in 6 to 1 in 10, as is the case when 1 c.c. of blood is added to an ordinary broth tube, the percentage of positive results is considerably smaller, and even when a positive result is obtained the culture has to be incubated for a much longer period before the growth can be demonstrated. The obvious disadvantage of this method is the very large amount of medium that is necessary.

(ii) A definite advance, as regards this group of organisms, was made when Conradi introduced the use of sterilized ox-bile as the medium into which the blood is inoculated.

Here again it has long been known that bile not only inhibits the clotting of blood, but also destroys all its antibacterial properties. A mixture of blood and bile also makes a very fair medium for the growth of bacilli of the typhoid-coli group, but it inhibits the growth of, or in some cases actually kills, many of the cocci and other bacteria. By using ox-bile in making the blood cultures, the diagnosis of enteric fever and allied diseases during their early stages has been greatly simplified.

In making such cultures 1 part of blood is added to two parts of sterilized bile in a test-tube. The bile causes complete haemolysis, and the resulting mixture consists of a thick opaque fluid. As it is difficult to ascertain if growth has occurred except by subculture, the usual procedure is to incubate the mixture for 18 to 24 hours at 37° C. and then to make subcultures on agar slopes. The resulting subcultures can then be examined microscopically and by other means, and the bacillus may sometimes be definitely diagnosed in as short a period as from 60 to 72 hours after making the blood culture.

In lieu of ox-bile certain solutions of bile salts, mixtures of glycocholate and taurocholate of soda, have been used. These solutions have given very similar results.

(iii) More recently a medium has been introduced by Douglas and Colebrook [1] which consists of broth to which a sterile solution of trypsin has been added. The reason for proposing this was that trypsin when in sufficient concentration has been shown to destroy all the antibacterial properties of the blood; further, such a concentration completely inhibits the clotting of the blood, neutralizes the 'antitryptic' power, and destroys any inhibitory

[1] Douglas and Colebrook, *Lancet*, 1916, ii, 180.

power the blood fluids may possess in regard to the growth of bacteria. Lastly a mixture of blood and trypsin forms a more than usually good medium for the growth of bacteria.

Experiments, both on man and animals, have shown that when the blood is cultivated in this medium the resulting growth occurs more rapidly and in a larger proportion of the tubes than in control cultures made in broth to which no trypsin has been added. On several occasions positive cultures have in fact been obtained in the broth containing trypsin when no growth has occurred in the tubes to which no trypsin had been added. The most convenient preparation of trypsin for this purpose is Inj. Trypsinae Co. (Allen & Hanbury), which is also now labelled 'Trypsin for bacteriological uses'. This preparation should be sterile and constant in strength. It should be added to the broth in the proportion of 1 part of trypsin to 20 parts of broth. When 1 c.c. of blood is added to 5 c.c. of such broth clotting is completely inhibited even in those cases where the 'antitryptic' power is very much above the normal and all the antibacterial properties of the blood are thereby completely destroyed.

It is evident that any sterile trypsin solution may be substituted for that recommended above, provided that it is of equal strength.

It is advisable to test the sterility of the trypsin as soon as it is received from the manufacturers. This is most conveniently done by making a 5 per cent. solution in broth, distributing this into tubes, each of which should contain at least 5 cubic centimetres, and then incubating the tubes at 37° C. for 48 hours. If the tubes show no signs of contamination they are stored in a cool dark place and used as occasion arises for making blood cultures. The diluted trypsin retains its properties for several weeks.

Even if the trypsin solution is found to be contaminated it may be sterilized by the method advocated by Fildes and Rajchman for albuminous fluids. This method consists of adding 3 per cent. of pure sulphuric ether or 0·25 per cent. of chloroform to the trypsin solution, which should be placed either in a stout bottle capable of being securely corked or in a strong serum bulb which can be sealed in the blowpipe flame. The vessel containing the etherized trypsin is then placed in a water bath at 48° C. for one hour, and after being cooled is opened up and the ether or chloroform driven off at a temperature not exceeding 55° C. This method gives very reliable results when the contaminating organisms are not spore-bearers, but when these latter are present the results are not constant.

A proportion of the cultures made should always be grown anaerobically, as some micro-organisms, for instance streptococci, develop more rapidly in the absence than in the presence of oxygen.

(iv) Another excellent method, as it gives a certain amount of information as to the number of bacteria present in the blood, is to plant measured quantities, for instance 0·1 c.c., 0·25 c.c., and 0·5 c.c. into about 25 c.c. of agar that has been melted and cooled to 45° C., thoroughly mixing, and then either allowing the agar to set so that a shake culture is made or pouring the contents of the tubes into Petri dishes. After incubation any colonies that develop can be subcultured, and by estimating their number some

idea of the number of bacteria present in a given volume of blood may be gained.

Skin contaminations.—In making blood cultures contaminations occasionally occur. These vary in numbers with the technique employed in collecting the blood, but are very uncommon when a syringe or such an apparatus as a Keidel's tube is employed in the hands of a practised operator.

Contamination may be suspected (i) when growth occurs in one tube only of a series, (ii) when different bacteria grow in different tubes, (iii) when a mixture of bacteria is found in the same tube, or (iv) when it is inherently improbable that the organism recovered was present in the blood stream. No one of these conditions proves the organism to be a contamination, but should always suggest caution in drawing any conclusion. One of the commonest contaminating organisms in blood cultures is the white staphylococcus known as *Staphylococcus epidermidis albus*, a common denizen of the skin.

(d) *Transmission.*

There is no doubt that the best course is to make the cultures at the bedside; unfortunately this cannot always be done and the blood has to be sent either by post or by messenger to the laboratory.

To transmit the blood in a sterile tube without further treatment is the least satisfactory method, for the blood will arrive in a clotted condition from which it is very difficult to obtain cultures. Further, any bacteria it may contain will have been liable to destruction by the antibacterial properties of the blood. But even under these unfavourable circumstances successful cultures may sometimes be obtained, small pieces of the clot being planted into various media. It is unnecessary to emphasize that the tube must be sealed hermetically or closed with a sterile rubber bung. It should never be closed with cotton wool or sealing wax.

If the presence of members of the enteric group of bacteria is suspected it is well to distribute part of the blood into tubes containing sterile ox-bile, the remainder may be placed in a tube containing ammonium oxalate solution[1] or dealt with by one of the methods mentioned below. A solution of citrate of soda has the same effect as has the ammonium oxalate, but appears to prevent the growth of many bacteria and should therefore not be employed.

Another useful method is to mix the blood with sterile melted gelatine which is then allowed to set. The proportion of blood to gelatine should be about 1–4.

Lastly, another method recently introduced is that of mixing the blood with a sterile solution of trypsin of sufficient strength

[1] A satisfactory formula is that recommended by Ryttenberg (*J. Med. Research*, 1909, 20, 79):

Ammonium Oxalate 2 grammes,
Sodium Chloride 6 grammes,
Aq. destil. 1000 c.c.

The resulting solution to be distributed into large test-tubes in quantity of 10 c.c. Sterilize for 20 minutes on 3 days in succession.

10 c.c. of ammonium oxalate solution is more than sufficient to decalcify 10 c.c. of blood.

to inhibit clotting. Using the Inj. Trypsinae Co. (Allen & Hanbury), one part of trypsin should be added to 4 of blood; this mixture may be incubated without further dilution as it forms an excellent culture medium.

In those cases when only Wright's capsules are available, it is best to run in some of the oxalate or trypsin solution before drawing the blood, and some more after the blood has been added. The capsule is then sealed in the usual way and the contents are quite easily mixed by gentle shaking.

III. General Considerations regarding Methods for the Isolation of the Pathogenic Bacilli.

The culture media which are employed in the isolation of specific bacteria from the intestinal contents vary to some extent with the organism for which search is made. For instance, the precise reaction needed is not always the same, and care must be taken that this is adjusted to the purpose in view; again as regards the presence of inhibitory substances in the medium, the enteric group of bacilli tolerate these better than members of the dysentery group, but there is probably no differential medium on which these bacteria will grow so certainly as on plain agar. In discussing the most suitable media for each group attention will be drawn to these points; the precise formulae for the media will be found in an appendix; here the general principles of the subject are alone discussed.

Even in enteric fever the vast majority of the organisms present in the faeces are, commonly, harmless saprophytes, usually outnumbering the pathogenic agent by several hundreds to one. On ordinary media, such as agar or gelatine, the colonies of many coliform bacilli, not specifically pathogenic, so closely resemble those of *B. typhosus* and *paratyphosus* that, although these media are perhaps the most suitable for ensuring the growth of the pathogenic forms, the labour involved in testing the large number of colonies produced would be too great. Two different plans for overcoming this difficulty are in use. In the first, a 'differential medium' is employed which renders the colonies of the pathogenic form different in appearance from those of the ordinary coliform bacilli, thus greatly restricting the number of colonies which need to be tested. Cultures from the faeces are made directly on to such a medium. In the second a preliminary cultivation is made in a so-called 'enrichment medium' which permits the growth of the organism sought, while inhibiting that of other organisms; from this sub-cultures are made on the differential medium.

(1) Differential Media.

It is fortunate that nearly all the pathogenic bacteria found in the faeces are unable to ferment lactose, while the great majority of the harmless organisms actively ferment this sugar, with production of acid. If, therefore, lactose be added to an agar medium containing no other sugar, and at the same time an indicator be added to reveal the change which has taken place, the lactose-

fermenting colonies will appear of a different colour from those which do not ferment it—a difference often accentuated by the actual production of alkali on the part of the latter. This principle forms the basis of nearly all the differential media in use. The number of indicators which can be used is very large. Litmus is very commonly employed, but is not satisfactory. There are many anilin dyes such as neutral red, basic fuchsin, malachite green, &c., which show more distinctive changes with acid and alkali and which are to be recommended in preference.

Another principle which can be employed lies in introducing into the medium some substance which prevents the growth of bacteria other than those sought. Thus bile salts permit the growth of bacilli of the colon group, but inhibit staphylococci and many streptococci. Again, there are certain anilin dyes, e.g. crystal violet, which in certain concentrations will allow of the growth of the enteric group and coliform bacilli and inhibit many other organisms.

One of the simplest of differential media consists of ordinary agar containing 1 per cent. lactose and tinted with litmus. On this the lactose fermenters yield pink colonies, while those of the non-lactose fermenters are blue. The well-known medium of v. Drigalski and Conradi, though not an easy one to prepare, is a development from this in which nutrose is added, together with one part in 100,000 of crystal violet. An excellent and widely used medium is that devised by MacConkey, in which bile salts are added to lactose agar, and the whole tinted with neutral red: here the dye becomes intensified, as it were, in the lactose-fermenting colonies, making them brilliant crimson, while the non-lactose-fermenting colonies are pale. Much the same happens when malachite green is used as the indicator. In Endo's method the medium is coloured with basic fuchsin partially bleached with sodium sulphite: on this the lactose-fermenting colonies are of an intense crimson, the others colourless.

It must be remembered that all these differential media merely reveal the presence or absence of lactose fermentation. Amongst the bacteria not fermenting lactose are many not uncommon intestinal species, such as *B. faecalis alkaligenes* (Petruschky) and the various so-called 'paracolon' bacilli which still have to be excluded by later tests. Moreover, some organisms ferment lactose very slowly; these colonies at first resemble non-lactose-fermenters on a differential medium.

The principle of introducing other sugars into the medium in order to render it more sharply differential may indeed be extended widely, the sugar or sugars employed being always such as are not fermented by the organisms sought for but are fermented by those which are not.

For instance, Russell recommended a medium containing 1 per cent. lactose and 0·1 per cent. glucose, litmus being employed as an indicator. This medium was modified by Krumwiede and Kohn, who advocated a medium containing 1 per cent. lactose, 1 per cent. saccharose, and 0·1 per cent. glucose, Andrade's indicator[1] being

[1] This consists of a 0·5 per cent. solution of acid fuchsin bleached by the addition of normal caustic soda solution.

used in place of litmus. Media with Andrade's indicator are unsuitable for plates and are employed in the following manner:

The medium, usually containing 1 per cent. lactose and 0·1 per cent. glucose and 1 per cent. Andrade's indicator, is distributed into tubes in such quantities that when sloped a butt of solid agar one inch deep remains at the bottom of the tube. When a suspicious colony is to be examined, transplant it on such a tube, making a stab into the butt and a streak along the sloped surface. In 24 hours, if the organism is a frank lactose fermenter, the whole agar to the top of the slope will be red. If a glucose but not a lactose fermenter, the red colour will be apparent round the stab only. If not a glucose fermenter no colour will develop. If gas formation occurs it is readily apparent.

Lastly a medium has been lately introduced by Holt-Harris and Teague,[1] which consists of nutrient agar containing a mixture of cane sugar and lactose, coloured by the addition of solutions of eosin and methylene blue. On this medium the common faecal bacteria give almost black colonies, while those of the enteric group are practically colourless, and are, in consequence, very easily seen on the reddish-purple background formed by the medium.

It is important to note that if lactose-fermenting bacteria are present the acid which they produce will inhibit the growth of vibrios of the cholera group.

(2) Enrichment Media.

The term 'enrichment' may be applied when, in a mixture of bacteria, a particular type of organism is caused to increase in numbers relatively to the rest. Thus a culture can be obtained which, as compared with the original material, contains the organism under consideration in much greater abundance or perhaps in the pure state. Such enrichment can be effected by a variety of agencies, physical and chemical, which may either produce a concentration of the specific bacteria present, or which may have the effect of retarding or preventing the growth of the concomitant organisms, while at the same time they permit, and perhaps actually favour, the proliferation of the particular bacteria. Antiseptic compounds which exert a relatively powerful inhibitory action on one group of organisms as compared with another are often used for the purpose; these may be termed 'differential antiseptics'. In the case of an admixture scanty in bacilli of the enteric group with abundant coliform organisms, such as occurs commonly in faeces in the 'enteric' fevers, a method of enrichment of the former would prove of great value. The property of effecting such enrichment when added to nutritive media has been attributed to several substances, e.g. caffein, but without much justification. v. Drigalski and Conradi, however, pointed out that certain dyes of the diamino-triphenylmethane group (malachite green and brilliant green) when present in suitable concentration in a culture medium inhibit the growth of the ordinary types of coliform bacilli, while permitting *B. typhosus* to proliferate actively. However, the earlier attempts to utilize this property for the

[1] Holt-Harris and Teague, *J. Infect. Dis.*, 1916, **18**, 596.

purpose of isolating *B. typhosus* did not appear to be any improvement over the method of plating on any of the recognized differential media. This was in great part due to the fact that solid media were employed, and, in consequence, the specific bacilli, when scanty, were planted in the midst of numerous other organisms contained in the faeces. Now it has been ascertained that the inhibitory effect of a given concentration of the reagent is overcome when sufficiently large numbers of organisms are used for inoculation; hence if many coliform bacilli were present in the sample planted they would be able to proliferate in spite of the antiseptic on that portion of the plate where the scanty typhoid bacilli were situated, and these latter would in consequence be overgrown. A more suitable method is to employ a fluid medium in which the mixture of organisms is uniformly subjected to the action of the differential antiseptic compounds.

It has been found that the differential action of brilliant green on *B. coli*, as compared with *B. typhosus*, is more marked than that of malachite green; hence the former is preferable as an enriching agent. Paratyphoid and Gärtner bacilli are even more resistant to these substances than is *B. typhosus*; thus brilliant green enrichment is specially useful in the case of paratyphoid infections. Certain types of coliform bacteria are even more resistant to brilliant green than the typhoid-paratyphoid group; it is therefore advantageous at times, in addition to certain concentrations of brilliant green, to add a solution of telluric acid which has the property of inhibiting a number of the bacteria, but permits the pathogenic group to proliferate. This method is of no value for the isolation of the dysentery bacilli.

Another method depending on the action of a differential antiseptic is that described by Bierast,[1] which consists in treating the specimen of faeces with petrol ether. The result is that organisms of the coli group can be killed while typhoid and paratyphoid bacilli survive and can be recovered from cultures of the treated faeces. Certain strains of *B. coli*, however, are resistant. Hall[2] has confirmed Bierast's work; he recommends the following procedure: a portion of faeces of the size of a pea is emulsified as uniformly as possible in a test-tube with 7–8 c.c. sterile broth; the emulsion is poured carefully into a thick glass tube 3–4 cm. in diameter and 10–15 cm. high, any unemulsified masses of faeces being kept back; then 4–5 c.c. petrol ether (b.p. about 40° C.) are poured in and the tube is closed with a tightly fitting indiarubber cork. The tube is shaken vigorously for half an hour (about 150 vibrations per minute) and thereafter allowed to stand for 1½–2 hours at room temperature.

A portion of the material at the foot of the tube is then withdrawn with a sterile Pasteur pipette and one drop is used to inoculate a plate of solid differential medium.

According to Hall the method is not applicable for the isolation of 'paradysentery' and cholera bacilli. A great advantage of the use of petrol ether is that it destroys *B. proteus*.

[1] Bierast, *Centralbl. f. Bakt.*, i Abt. Orig. 1914, 74, 348.
[2] Hall, *Berl. klin. Wchnschr.* 1915, 52, 1326.

In any event direct plating of the stool should invariably be carried out in addition to the use of enrichment methods.

(3) Cultures from Faeces.

Cultures should be made from faeces as soon as possible after evacuation, as when specimens are allowed to stand for some time the isolation of the specific bacilli becomes increasingly difficult. When delay is unavoidable, the glycerine preservation method (see p. 21) should be employed.

The material for culture is spread over a considerable surface of solid medium in Petri capsules, so as to obtain *isolated* colonies. In the case of faeces, where the non-specific organisms may be very numerous, the aim is to secure a large number of discrete colonies —at least several hundred to a thousand or more, as it has been estimated that 1 : 300 represents the lowest proportion of typhoid bacilli which will yield positive results by direct plating.

Preparation of Plates.—In the making of plates for cultures it deserves note that to obtain proper differential effects the layer of coloured medium should not be too thin ; it should be at least 2 to 3 mm. in thickness in the centre of the plate. Further, care has to be taken to secure a proper dryness of the surface of the medium, as if this is neglected the colonies tend to run together and to form a bacterial film which renders the isolation of the individual colonies impossible. In order to obtain this result the plate should not be poured until the agar has cooled to 45° C., and after the medium has solidified, the agar-containing half of the Petri dish is placed, with its glass surface uppermost, in the incubator, one edge resting on a piece of glass tubing, which also may be used to support the cover. It is well to have under both a sheet of clean paper. In general the proper degree of dryness can be reached in from half to one hour. This process of drying may be undertaken either before or after inoculation. Another device consists of the introduction of some absorbent material, such as a disk of filter paper, into the lid of the capsule. A method, which has been in use for years, consists in covering the freshly poured plate with a sheet of sterile lint stretched by means of two copper or brass rings, fitting closely one inside the other, the inner being large enough to cover the part of the dish containing the agar. The whole process is conducted in the incubator. When the plate is dry the lint cover is replaced by the lid of the dish. When this method is employed the agar may be poured even when boiling.

Several different methods of making the inoculation have been suggested, e.g. (1) a drop of liquid faeces, or of an emulsion of the solid faeces, is placed on the surface of the plate and is spread all over with a piece of glass rod bent at a right angle ; the rod is then used to spread a second and possibly a third plate in succession, so that, according to the number of organisms present, one or other of the plates may be expected to yield abundant discrete colonies ; for this purpose at least two or three 3–4 inch Petri plates must be employed for each specimen ; (2) a second procedure consists in placing a loopful of fluid faeces or faecal emulsion on the medium at one side of the plate ; continuous strokes are then made (see Figs. 3, 4 and 5), bringing the loop in flat contact with a considerable area of the medium ; by this means a single plate

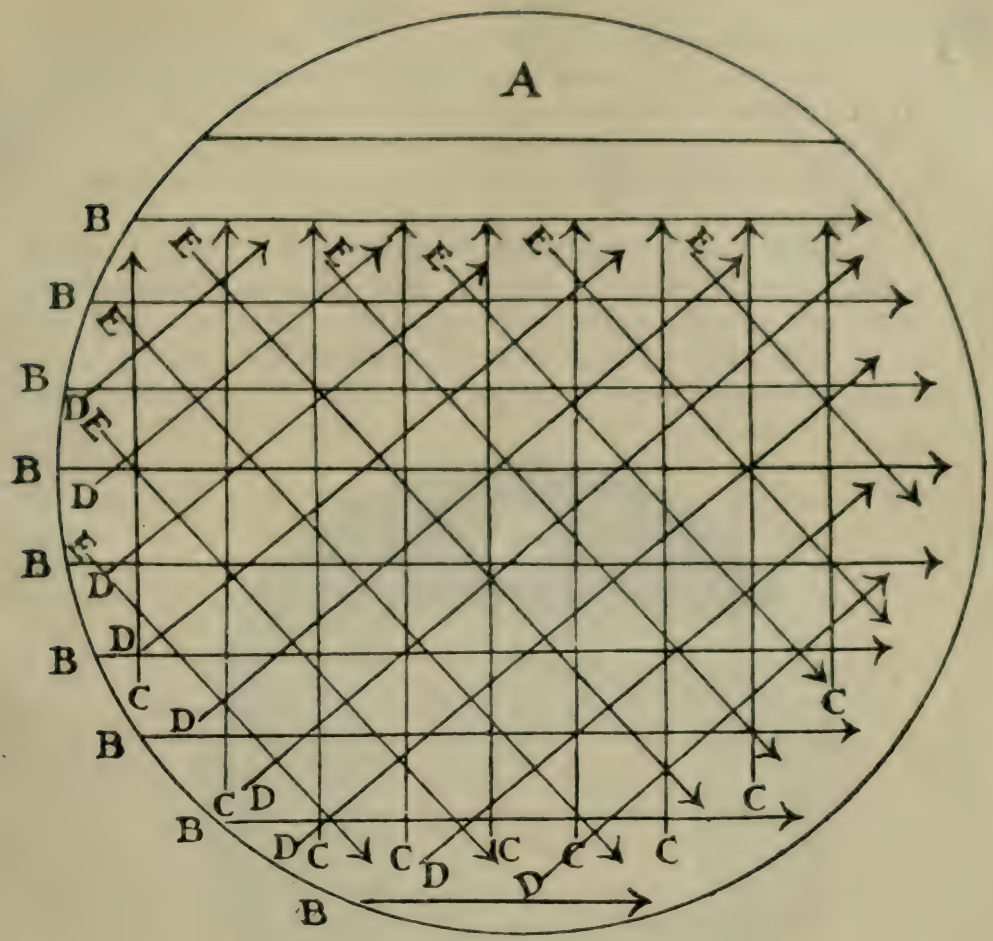


Fig. 3.

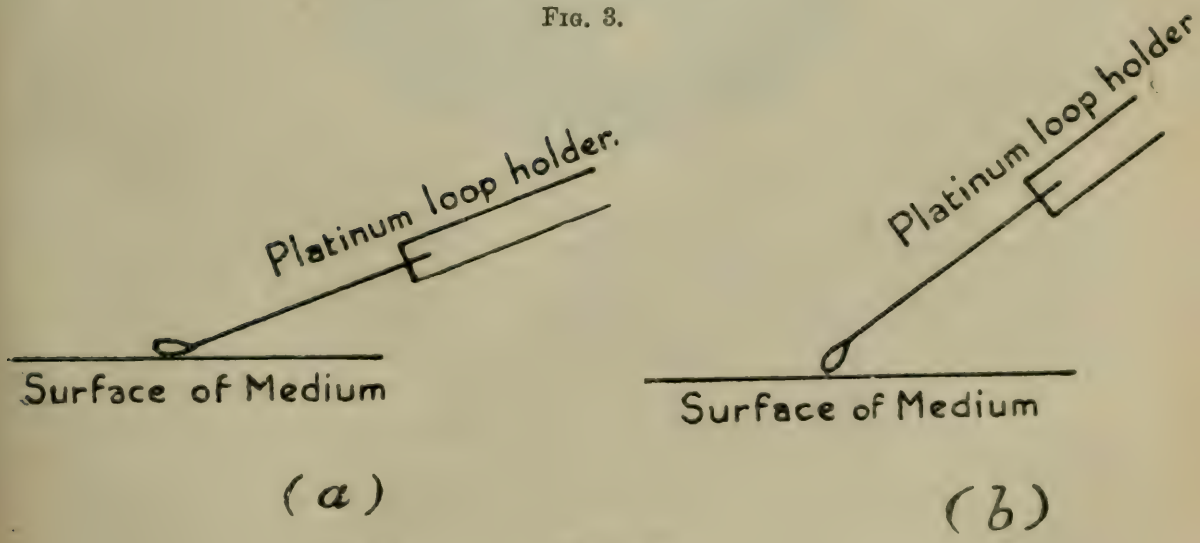


Fig. 4.

Figs. 3 and 4. Diagrams to illustrate the method of inoculating a plate with faeces.

A loopful of the specimen (fluid faeces or an emulsion of solid faeces) is rubbed over the area A. The loop, having been sterilized in the flame, is then rubbed over the area A and when charged in this way is used to inoculate the remainder of the plate by successive series of strokes, viz. B, C, D, and E, which are made in the directions indicated by the arrow heads. The needle should be held throughout so that the whole of the loop comes into contact with the surface of the medium as shown in Fig. 4 *a* (not as in Fig. 4 *b*), since by this means the colonies which develop are more evenly distributed over the plate.

will usually suffice for each specimen, especially if an enrichment culture is made at the same time.

A number of workers consider that more successful results are obtained if the faecal emulsion in saline is allowed to stand for half an hour at room temperature, or an emulsion in bouillon is placed in the incubator for several hours and then cultures are made from the upper layer.

The plates are examined after incubation for 12–24 hours at 37° C.; all lactose fermenting colonies may then be disregarded as being certainly not the pathogenic organisms. Several suspicious colonies are picked out for microscopic examination and for sub-culture on agar and further tests. At least three such colonies

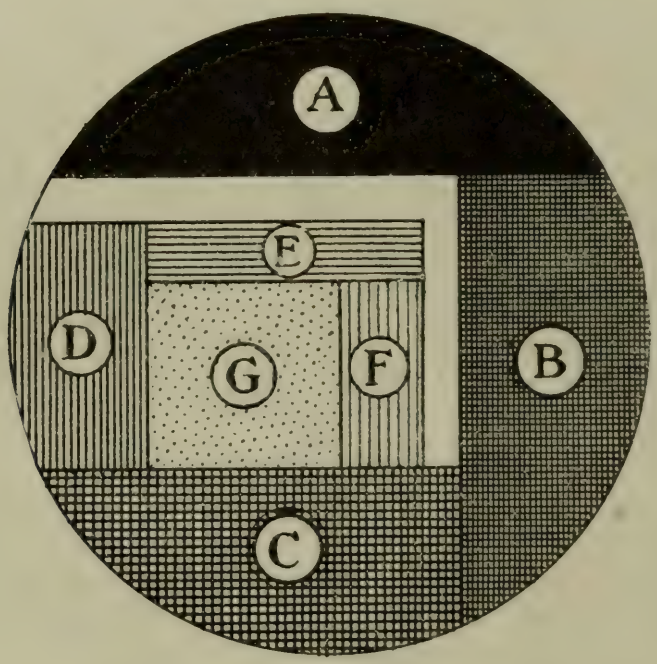


Fig. 5. A loopful of the faecal emulsion or mucus is spread over the area A with a platinum needle which is then heated to remove any excess of material, and when cool, again rubbed over A. Then, with the loop in flat contact with the agar surface, the areas B, C, D, E, F, and G are in turn inoculated without again heating the platinum loop. In this way each area is inoculated with a diminishing quantity of the material, so that one portion or another of the surface will give a number of separate colonies.

should be selected, those of different appearance being chosen if more than one type is present.

In selecting colonies no great reliance should be placed on their naked-eye appearance, as this is a variable character. In the case of *B. paratyphosus B* colonies, however, a striking feature consists in the formation of a raised opaque border, which develops when the plate culture, after incubation for eighteen to twenty-four hours at 37° C., is allowed to remain for a further period of twenty-four hours at room temperature. This character, while not absolutely specific for *B. paratyphosus B*, is of great value and enables it, when present in scanty numbers, to be picked out from a plate culture so rich in other non-lactose fermenters that, otherwise, it would in all probability have been missed.

Some workers employ a rough agglutination test at this stage, rubbing up a trace of a colony in a drop of saline on a slide and adding a drop of a low dilution of one or more potent antisera. This procedure is useful for rapid work but is not wholly reliable and is best deferred for twenty-four hours, when the growth on an agar slope may thus be tested with far more satisfactory results.

Technique employed in using the brilliant green enrichment method.—At the same time as the direct plate is spread, a 10 c.c. tube of peptone water containing brilliant green 1 : 200,000 is inoculated with one or more (up to half a dozen) loopfuls of faecal emulsion and incubated at 37° C. After nine hours' incubation in the case of heavy inoculation, or after eighteen to twenty-four hours after light inoculation, subcultures in the form of three successive strokes on a plate of MacConkey's or other differential medium are made from the brilliant green culture. Rarely a culture is not obtained from the brilliant green tube till after 48 hours' incubation. In positive cases a practically pure growth of the typhoid-paratyphoid organism frequently results, so that there is a great saving of the time ordinarily spent in testing a number of likely colonies; also, the specific organisms, especially paratyphoid bacilli, are often recovered by this means when direct plating fails to reveal their presence. It is to be noted that the direct plate culture should never be omitted, as sometimes it may give positive results where the enrichment medium fails.

Direct plating together with enrichment in a single tube of brilliant green peptone water probably represents the procedure which will yield a maximum number of positive results with a minimum of labour. Owing to the fact that the conditions necessary to favour the growth of the specific organisms in a mixture of organic material and other bacteria, such as faeces, depend on a number of variables, it is certain that the specific organisms will be missed by the above procedure in a considerable proportion of cases, from which they could be recovered if several concentrations of brilliant green were employed with and without the addition of telluric acid, viz. 1 : 400,000 and 1 : 200,000 brilliant green with and without telluric acid 1 : 25,000, or if brilliant green be employed alone three strengths (1 : 400,000, 1 : 250,000, 1 : 150,000) should be used. By adopting glycerine preservation the specimen will be available for this extended investigation should the preliminary examination prove negative.

The incidence of the enteric group of bacilli in the faeces.—*B. typhosus* has been found most frequently in the first three weeks of the illness, especially the third week. In paratyphoid infections the maximum occurs earlier—in paratyphoid A in the first and second weeks (Krumwiede), especially about the twelfth day, and in paratyphoid B cases at the end of the second and beginning of the third weeks; thus the bacilli appear to be most readily recovered about the commencement of defervescence. While these are the periods to be chosen if only a single examination is made, repeated tests should if possible be carried out at short intervals in all cases until the diagnosis is established. *B. enteritidis* is usually isolated from the diarrhoeal faeces in the acute attack.

(4) Cultures from Urine.

The specimen of urine may be centrifugalized and successive stroke cultures made from the sediment on a plate of MacConkey's, Endo's, or v. Drigalski and Conradi's medium; the subsequent procedure is then the same as that outlined above. When the specific organisms are scanty, centrifugalization and cultivation from the sediment probably does not represent the best method; it is then preferable to incubate a comparatively large volume of the urine. Morishima and Teague find, however, that certain urines inhibit the growth of *B. typhosus*, but that this inhibition is overcome by adding two volumes of broth to one of urine. Owing to the liability of specimens of urine to contamination, especially with coliform organisms, brilliant green, to make a concentration of 1:250,000, may be added with advantage to a duplicate specimen prior to incubation.

The value of bacteriological examination of the urine in cases of typhoid-paratyphoid infection merits more extensive recognition. It is an especially valuable diagnostic procedure when repeatedly carried out, as there appear to be transient bacilluric periods in enteric infections; hence a simple daily examination performed by plating out two or three loopfuls on a MacConkey plate over a period of seven to ten days will lead to the isolation of the infecting organism from a large proportion of patients. Thus in a series of twelve test cases examined during the second and third week of illness, the specific organism was recovered in each by this means.

(5) Cultures from Blood.

The blood culture in bile, bile salt medium, or trypsin bouillon, should be examined at daily intervals for at least a week. These examinations should be made macroscopically whenever possible; frequently, especially in the case of cultures in bile, it is necessary to make a daily subculture, as it is impossible to ascertain if growth has occurred by naked-eye examination. In all cases before a tube is discarded, subculture should be made, whether growth is suspected or not.

The proportion of positive blood cultures depends directly on the stage of the disease at which the specimen has been taken, as has been already emphasized; thus, in cases not previously inoculated against the infecting organism, isolation of the specific bacillus is most likely to prove successful at the commencement of fever, during the first week with *B. typhosus*, and especially in the earlier part of the first week with the paratyphoid bacilli. Provided the cases are examined within the first week, blood culture will be almost uniformly successful in leading to the detection of the enteric group of infections; thus in a series of upwards of 130 cases of acute febrile illness J. F. Smith[1] obtained thirty-four positive results, and of the remainder no single one was subsequently proved to be suffering from a typhoid-group infection in spite of extended bacteriological and serological investigations. In cases which have previously undergone inoculation with the infecting

[1] Browning, *Applied Bacteriology*, 1918, p. 43.

organism, it has been stated that blood cultures are frequently negative, but this is by no means necessarily the case. In *relapses*, especially at the commencement, the organisms may also be obtained from the blood.

(6) Cultures from other Secretions and Pathological Products.

Organisms belonging to the enteric group may be recovered from bile, pus, and occasionally from the stomach contents or sputum. Where there is reason to suspect a mixed infection, preliminary plating on a differential medium should be practised as in the case of faeces.

IV. The Specific Diagnosis of the Various Bacterial Infections.

(1) The Enteric Group.

One or more suspicious colonies from plates prepared by one of the methods described in the previous section will have been subcultured on agar. In order that such a culture should be identified as one of the pathogenic members of the group it must possess a series of biological characters some of which, e.g. form and staining, are common to the group, while others are more or less specific, viz. fermentation and immunity reactions. The main characters on which dependence is placed are as follows:

(a) *Morphological characters*, including motility and staining:

(b) *Biochemical reactions*—(i) fermentation tests, i.e. the determination whether certain sugars or alcohols, introduced into the media, are broken down, during the growth of the bacilli, with the production of acid or acid and gas; (ii) effect on milk to which an indicator has been added; (iii) production of indol from peptone water; (iv) liquefaction of gelatine:

(c) *Immunity reactions, especially agglutination.* When examining a culture it is advisable to observe a certain sequence in the procedures, since an organism which fails to conform to particular criteria can be rejected at once without loss of time being incurred in performing further tests. Thus it is important to determine first of all that the culture consists solely of Gram-negative bacilli; this being decided, one proceeds to examine for motility, then the biochemical reactions are tested, and finally the agglutination test is made.

(a) *Morphological characters and staining.*

All organisms of the enteric group are Gram-negative non-sporing bacilli. Young cultures (up to 24 hours at 37° C.) as a rule stain uniformly with dilute carbol-fuchsine, although the protoplasm may fail to stain uniformly with methylene blue. The bacilli vary considerably in length and thickness; in cultures of *B. typhosus* long forms tend to be frequent, but no diagnostic importance can be attached to this feature.

Motility may be tested by examining a young broth culture (4 to 24 hours), or better still by inoculating a hanging drop of broth with a minimal trace of the organism, sealing with vaseline,

and incubating at 37° C. The preparation should be examined from hour to hour up to 6 hours. Dark-ground illumination is useful, but not essential, in examining for motility. In order to determine that an organism is motile, definite movements of bacilli across the microscope field in various directions must be observed; this must be distinguished from (1) streaming movement where all the organisms flow in one direction, and (2) brownian movement, i.e. oscillation to and fro and around a given point, but with no definite translation. Organisms which are motile, when suitably stained, show flagella, which in this group are situated all round the bacilli; little diagnostic importance attaches to the number or length of the flagella. Occasionally a strain of *B. typhosus* may show no motility for a long period after isolation.

(b) *Biochemical reactions.*

(i) *Fermentation tests.* The substances commonly employed for these tests in the enteric group of bacilli are glucose, lactose, saccharose, mannitol, and dulcitol. The tests are made by inoculating fluid medium, containing a suitable amount of the reagent (usually 1 per cent.) and an indicator, put up in fermentation tubes. The use of solid media is not recommended as a routine practice.

(ii) In testing for the production of indol, cultures are made in peptone water, which are incubated at 37° C. and tested at intervals for the presence of indol.

(iii) In order to determine whether gelatine is liquefied, a stab or stroke culture is kept at room-temperature or in the cool incubator at 20° C. Another method is to incubate the culture at 37° C. and then remove it to room-temperature; in the latter case, if liquefaction has occurred, the medium will not become solid on cooling. Liquefaction usually begins to be apparent in 3–4 days at 20° C.

The principal reactions are shown in the following table:

Fermentation Reactions of the Typhoid-Paratyphoid Group.

Bacillus.	Glucose.	Lactose.	Saccharose.	Xylose.	Arabinose.	Mannitol.	Dulcitol.	Salicin.	Litmus Milk.
B. typhosus.	A	–	–	A or –	– or A	A	– or A	–	sl. A
B. paratyphosus A.	AG	–	–	–	AG or A	AG or A	AG or A	–	A*
B. paratyphosus B. *B. paratyphosus C.* *B. aertrycke* *B. enteritidis (Gärtner).*	AG	–	–	AG	AG	AG	AG	–	Alk. after initial A
B. suipestifer	AG or A	–	–	AG or A	– as a rule	AG or A	AG A or –	–	Alk. after initial A

All the above organisms are negative to indol and fail to liquefy gelatine.

AG = acid and gas. – = no change.

A = acid. Alk. = alkaline.

* May become alkaline after several weeks' incubation.

If at the end of 24 hours an organism appears to conform to one of the specific pathogenic types the agglutination reaction may be tested without further delay, but the fermentation tubes should be incubated for at least a week before making the final readings.

The fermentation reactions of a member of the enteric group should not be regarded as definitely determined until the organism has been grown on artificial culture medium for several generations. Thus the capacity to ferment certain substances, e.g. lactose, is not manifested by some organisms until they have become habituated to artificial culture media, and both time and repeated subculture are factors in bringing this about. Conclusions based on the behaviour of newly isolated organisms may be responsible for troublesome errors in this respect; usually, however, three or four subcultures extending over a period of a week altogether will enable the stable characters of the culture to develop.

It is of practical importance to differentiate (1) weak fermentative characters, which may be manifested by recently isolated organisms, and are comparatively soon replaced by more stable characteristics, and (2) mutation involving the acquisition of power to act on a given substance after more or less prolonged growth on medium containing the particular carbohydrate or alcohol.

(1) *Weak fermentative characters which may be exhibited by recently isolated organisms.* A strain which fails to ferment lactose immediately after isolation may be found to have acquired this property after several subcultures on agar. Accordingly there may be danger of regarding an organism as a non-lactose fermenter, and hence possibly a pathogenic type, if reliance is placed on the incomplete fermentative reactions exhibited immediately after isolation. On the other hand, caution must be exercised in discarding a newly isolated strain owing to its being deficient in the fermentative properties characteristic of a given pathogenic type, e.g. absence or deficiency of gas formation exhibited by some recently isolated strains of *B. paratyphosus A*. Attention to such points will obviate many fallacious conclusions, e.g. that paratyphoid bacilli form indol, or that an organism, otherwise resembling paratyphoid A, is to be discarded because it has not fermented dulcitol with gas formation.

(2) *Mutation* resulting from prolonged growth in a medium containing a given substance is not likely to prove a disturbing factor in practical work, except in the case of *B. typhosus*, which, as Penfold pointed out, may form acid in dulcitol media after growth for some days.

It is important to note that a freshly isolated organism may be deficient in fermenting power or motility and should not be immediately excluded on that account; on the other hand any *excess* of characters at once disqualifies it as a member of the genuine enteric group.

In addition to the series of tests indicated above, the use of certain other substances has been recommended as of value in particular instances. Thus (1) *B. typhosus* forms acid from sorbitol and so differs from Shiga's dysentery bacillus, which has not this property. This test is of value when a non-motile strain of *B. typhosus* has been isolated,

(2) Organisms are not uncommon in the intestines of animals and even of man, which, in their general cultural characters, resemble the pathogenic Salmonellas, though having no serological affinity with them. Such organisms may usually be distinguished by their power of fermenting *salicin*, which the pathogenic Salmonellas fail to do.

(3) All the members of the Salmonella group ferment *rhamnose*, and all, except *B. paratyphosus A*, ferment *xylose*.

When an organism conforms to one of the standard pathogenic types in biochemical reactions, its behaviour in agglutination tests should be examined by means of high-titre agglutinating sera derived from animals.

Some workers prefer to abbreviate the series of fermentation tests and consider that economy is effected in time or materials by passing at once to agglutination reactions. Thus the following procedure may be adopted where suspicious colonies have been obtained :

Abbreviated Scheme of Identification.

Stage (i). Inoculate one tube each of
(*a*) lactose peptone water with an indicator
(*b*) mannitol peptone water with an indicator
(*c*) sloped agar, including its condensation water
(*d*) a tube of broth
} and place at 37° C.

Stage (ii). Examine the condensation water of the agar culture or the broth tube after six hours for motility; return the cultures to the incubator.

Stage (iii). Next day observe (*a*) and (*b*)—
if (*a*) shows acid or acid and gas, reject the strain; if (*a*) shows no change and (*b*) shows acid only, test agglutination with anti-typhoid serum if the culture was found to be motile at stage (ii), and also with anti-dysentery serum if non-motile. (*Note* (1) an organism may occasionally not ferment lactose until later, and (2) *B. typhosus* is occasionally non-motile.) If (*a*) is unaffected and (*b*) shows acid and gas, test agglutination with para A, para B, and Gärtner anti-sera, and if necessary with a para C anti-serum also.

(c) *Serological Tests.*

The principles and practice of the agglutination test are so fully described in Part II of this Report that it is unnecessary here to do more than mention those points to which special attention should be paid in the diagnosis of the enteric group of bacilli.

Agglutinating sera for the members of this group, which are usually prepared from the rabbit, possess a high degree of specificity. In the case of *B. typhosus* there is no evidence to suggest the existence of distinct serological races: a typhoid serum prepared from any one strain will agglutinate all the strains of typhoid, any differences of titre depending merely on differences of intrinsic agglutinability shown by individual strains; such differences are rarely great. The same is true of *B. paratyphosus A* and probably of *B. paratyphosus B*. *B. paratyphosus C*, however, presents, so far

as our present knowledge goes, two varieties, one of which is agglutinated by paratyphoid C sera only, while the other is also agglutinated by paratyphoid B serum to a quarter or even a half of its full titre for paratyphoid B. The Aertrycke bacillus is so closely allied to *B. paratyphosus B* that the serum of either will agglutinate the other almost or quite to its full titre for itself. *B. enteritidis* (Gärtner), on the contrary, yields a highly specific serum.

It follows that the worker who is provided with a range of sera such as the above and of a potency of 1 in 2,000 to 1 in 5,000, or even less, is seldom left in doubt as to the diagnosis of bacilli of the enteric group. Apart from the inagglutinable strains immediately to be mentioned, a culture which does not respond to one or other of these sera may reasonably be supposed not to belong to the group, and if in addition it shows any abnormal biochemical reactions it may be excluded with confidence. Human sera from cases of natural infection should not be used for the purpose of diagnosing unknown bacilli.

Inagglutinable strains. Immediately after isolation a culture is sometimes more or less insusceptible to agglutination, and it may therefore be unsafe to base a final conclusion on deficient agglutinability until the organism has been frequently subcultured. Inagglutinability is said also to depend on certain substances, such as malachite green, which may have been added to the medium on which the organism has grown: the test is therefore better carried out with a subculture from such an original plate. In rare cases inagglutinability has been found to persist for a long period (McIntosh and McQueen[1]) and may be accompanied by lack of motility. If, then, an organism which resembles in all its other characters a member of the enteric group fails to agglutinate with suitable sera, it should not be rejected until it has been subjected to repeated subculture in broth. In obstinate cases the true nature of an inagglutinable strain may be revealed either by immunizing an animal with it, when the serum yielded may be found to agglutinate typical strains, or by means of the absorption test (see p. 137).

Co-agglutination. Trouble is sometimes experienced owing to the serum of one species agglutinating allied species to a certain fraction of its titre. Since this may occur, too much attention should not be paid to agglutination reaching only to a small part of the full titre of the serum for its own species. The degree, however, to which this co-agglutination is seen depends largely on the technique employed. It is agreed by all those who have employed formalinized broth cultures that the phenomenon is far less obtrusive than when suspensions of agar cultures in salt solution are used. A simple and reliable procedure is to pick off suspicious colonies into broth tubes, and after 24 hours' incubation to add to each broth tube an equal volume of a 0·2 per cent. solution of formalin in normal saline, shaking thoroughly. Such emulsions may be used for agglutination tests after standing for an hour

[1] *J. Hyg.* 1914, 13, 409.

at room temperature even though they are not yet completely sterile. A longer period is, however, desirable.

Much more rarely it has been observed that a specific serum will agglutinate bacteria having no relation whatever to the homologous species. This phenomenon, known as 'para-agglutination', is sufficiently discussed in Part II of this Report (see p. 99). It is usually a transient phenomenon, disappearing on subculture.

Discussion of Results. In testing an organism suspected to be a member of the enteric group, it should be put up against several dilutions of the requisite sera. The full titre of the serum being known, dilutions corresponding to $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, full, and even double titre are recommended.

(*a*) If the organism is agglutinated to the full titre of the serum its identity may usually be regarded as established.

(*b*) If it is agglutinated to $\frac{1}{4}$ or $\frac{1}{2}$ titre only there is a fair chance that it is identical with the organism employed in producing the serum, for it may be only a weakly agglutinable strain. This is especially likely to be so with a freshly isolated organism. On repeated subculture its agglutinability will, in such a case, probably increase, even up to the full titre of the serum. If this does not happen, the identity of the strain must remain in doubt.

A lesser degree of agglutination, to $\frac{1}{8}$ of the titre of the serum and no more, leaves the question still more doubtful, and the probabilities are against the identity of the strain with that used in preparing the serum. Nevertheless the expedient of repeated subculture should be tried.

(*c*) If the organism is not agglutinated at all by the serum, even after several subcultures, it is almost certainly not of the species corresponding. It may, in certain cases, belong to it, though inagglutinable, but this can only be demonstrated by the absorption test or by determining its agglutinogenic power. It may, again, belong to the species suggested by its fermentation reactions but to a special and perhaps unrecognized serological race.

(2) Bacillary Dysentery.

The methods which are applicable to the isolation and identification of the classical types of dysentery bacilli such as those of Shiga and Flexner are equably suitable for bacilli which are less commonly recognized as being the causal agents of allied conditions. The following instructions are, in consequence, of general application to the whole group.

In diagnosing bacillary dysentery or the carrier condition by bacteriological methods, the faeces are practically the only material which yields positive results. Blood-cultures yielding a growth of any of the dysentery bacilli have only very rarely been recorded, and the presence of these bacilli in the urine is almost equally uncommon.

(a) *Technique employed in making Cultures from Faeces.*

The technique employed for the isolation of these bacilli from the faeces is almost exactly similar to that employed for the isolation

of the enteric group of bacilli. It should, however, be remembered that the dysentery bacilli, as a group, are extremely sensitive to the action of the various anilin dyes and other reagents which have been employed to inhibit the growth of certain of the faecal bacteria. It is therefore advisable to use some simple medium such as lactose agar tinted with litmus, or some medium, samples of which have been personally tested and have been shown to have no marked inhibitory effect on the growth of bacilli of the dysentery group, e.g. MacConkey's medium, or one of the modifications of Holt-Harris and Teague's medium. The medium of v. Drigalski and Conradi is not to be recommended.

Up to the present time, no satisfactory enrichment method, such as the use of brilliant green in the case of the enteric group, has been discovered facilitating the isolation of dysentery bacilli; the plates have therefore to be made direct from the faecal emulsion.

In isolating the various members of the dysentery group of bacilli from the faeces it is even more important than in the case of the enteric group that the sample of faeces should be *as fresh as possible. Cultures should be made as soon as possible after the passage of the stool, and in no case should the interval be longer than six hours.* If the specimen cannot be examined at once, either the glycerine method of preservation (see p. 21) or that recommended by Dudgeon [1] should be employed. This latter method consists in thoroughly mixing the fresh specimen with an equal volume of a $\frac{N}{33}$ solution of sodium hydrate. Recent experience demonstrates that positive results are obtained with equal frequency when, instead of waiting for the passage of a stool, material is obtained direct from the lower bowel by one of the methods described below. The technique employed in obtaining cultures from a naturally passed stool will be first described.

Cultures from the stools. The technique employed in preparing the material for inoculating plates varies according as the examination is made (i) during the acute stage of the disease; (ii) during convalescence, or even later, to ascertain if the individual has a chronic infection or is a carrier of dysentery bacilli.

(i) During the acute stage of the disease, when the stools consist largely of blood and mucus, a portion of the mucus should be removed on a platinum loop and thoroughly emulsified in broth or peptone water, a procedure requiring considerable patience owing to the difficulty of breaking up the mucus. A convenient method of making a satisfactory emulsion is to take a test-tube containing a few cubic centimetres of sterile broth or peptone water, and by means of a platinum loop to place on the glass immediately above the surface of the fluid a portion of the blood-stained mucus. The mucus is then broken up by rubbing it with the platinum loop against the surface of the glass, and finally a very even emulsion is obtained by rubbing the fluid, loopful by loopful, into the broken-up mucus. The tube containing the emulsion having been allowed to stand upright for a short time in order that the coarser

[1] Dudgeon, *Med. Res. Com., Spec. Report Ser.* No. 40, 1919, p. 13.

particles may settle, the plates are then sown by any of the methods described above. (See p. 36 et seq.)

An alternative method is to place a small piece of the mucus directly on to the surface of the agar, and then by means of a platinum loop or glass rod to charge the plate or series of plates.

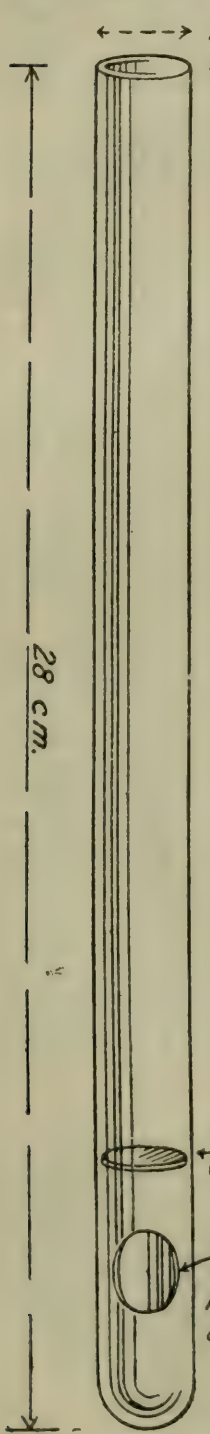


Fig. 6. Ziemann's Tube.

(ii) In those cases in which the patient is suffering from a chronic form of dysentery, or is suspected of being a carrier, (*A*) if blood-stained mucus is present a portion of this is picked out and the plates sown in a similar manner to that described above. (*B*) If, however, as is frequently the case, no mucus can be detected, and especially if there is a tendency to constipation, a mild purge may be administered. This, however, is less important than in the case of the enteric group, as here it is not the contents of the small intestine that are desired. A specimen of the loose stool, thus procured, may be examined by emulsifying a loopful or more of the faeces in broth or peptone water.

Cultures made from materials other than a naturally passed stool. These methods of obtaining cultures of dysentery bacilli have the advantage that the examination can be made at any time and quite independently of the passage of a stool. The following procedures may be mentioned:

(i) By means of the sigmoidoscope a swab may be taken from any ulcerated surface that is within reach. Experience has shown that such ulcerated surfaces frequently afford numerous colonies of the causal bacilli. This method is said to give very reliable results, but obviously it can only be employed in a limited number of cases, these as a rule being of the subacute type of the disease. In the acute stage the sigmoidoscope cannot be employed without a general anaesthetic, and in many chronic cases the examination fails to reveal any ulceration.

(ii) By means of rectal swabs. (See p. 21.) This method has been extensively used on the Western Front during the war, and the results during the acute stages of the disease have been quite as satisfactory as those obtained by examining a freshly passed motion.

(iii) By the administration of a plain water enema after the bowels have been opened and the utilization of the fluid thus obtained for

making the cultures. This method occasionally gives positive results when repeated examinations of the stools have failed. In obscure cases, especially those running a chronic course and when no causal agent can be discovered by other means, it is well worthy of a trial.

(iv) By means of a Ziemann's tube.

This consists of a glass tube 28 cm. long and about 2 cm.[1] in diameter, the glass walls being 1·5 mm. thick; three centimetres above the lower end, which is closed like a test-tube, is a circular opening 1½ cm. in diameter (see Fig. 6).

When a sample of the intestinal contents is required the rounded end of the tube is introduced into the rectum and pushed gently upwards with a rotary movement. Some of the mucus or faeces that are present in the rectum pass into the lumen of the tube through the lateral opening. The tube is then withdrawn and a sample of its contents removed through the lateral aperture. The apparatus is then washed, sterilized in cresol solution, again washed, and is then ready to be used afresh.

The author claims that this method is very rapid and easily carried out.

(v) Lastly, during an epidemic valuable information may at times be obtained by making cultures directly from the ulcerated portions of the large intestine during post-mortem examination carried out as soon as possible after death.

(b) *Identification of the Various Members of the Group of Dysentery Bacilli.*

The principles underlying the recognition of the bacilli capable of producing epidemic dysentery are similar to those governing the identification of the members of the enteric group (see p. 41). The main characters on which this differentiation rests are as follows:

(i) Morphological characters including motility and staining reactions.

(ii) Cultural and biochemical reactions consisting of:

- (*a*) Fermentation tests.
- (*b*) Effect on milk.
- (*c*) Production of indol from peptone water or some other suitable medium.
- (*d*) Liquefaction of gelatine.

(iii) Immunity reactions, especially agglutination and allied tests.

(iv) Acid agglutination.

It is unnecessary to describe these again in detail, and only those points which require special precaution need mention. It is unfortunate that all the bacilli capable of producing dysentery have not yet been completely investigated, and a final classification cannot at present be made. All authorities are, however, in agreement that the most important types which cause epidemic dysentery are those named after Shiga and Flexner.

[1] The exact diameter is not given, but from the diagrams given in the author's paper appears to be 2 cm. or a little more.

The characters of these two types will now be discussed, leaving to later in this section the description of the other bacilli which are suspected of causing dysentery.

After the plates of the selected differential medium have been incubated for 18 to 24 hours at 37° C. they are examined for the presence of any colonies which indicate by their colour that the bacilli forming them are unable to ferment lactose. The colonies of dysentery bacilli have no distinctive naked-eye characters by which they may be recognized from other non-lactose fermenting coliform organisms, but usually they are smaller and more transparent. After the plates have been examined and any suspicious colonies subcultured, it is always advisable to return them to the incubator for another period of 24 hours, the suspicious colonies if few in number, being marked by means of a spot made with a grease pencil on the glass. When the plates are re-examined a few extra colonies of non-lactose-fermenting bacilli are sometimes discovered, and these frequently prove to be members of the dysentery group, whose growth has been either very slow or inhibited during the first period of incubation.

The subcultures having developed, similar fermentation and other cultural tests are made as in the case of the enteric group of bacilli. The fermentation tests employed are those with glucose, lactose, mannitol, and dulcitol.

The following table gives the reactions in the case of Shiga's and Flexner's bacilli.

Bacillus.	Motility.	Fermentation test.				Indol.	Milk.	Gelatine.
		Glucose.	Lactose.	Mannitol.	Dulcitol.			
B. dysenteriae (Shiga)	–	A.	–	–	–	–	A.,	*No* liquefaction.
B. dysenteriae (Flexner)	–	A.	–	A.	–	+ or –	A., sometimes alk. later.	*No* liquefaction.

From this table it is seen that both these types of dysentery bacilli have quite definite and constant biochemical and cultural reactions by which they can be separated not only from the enteric group of bacilli but also from each other. If a culture gives all the characters of either of the two types it is highly probable that it is a true member of the dysentery group, but to make the identification certain, agglutination tests should be carried out.

In dealing with this group of micro-organisms, the method employed in making these tests should always be the macroscopic, the tubes being preferably placed in a water-bath at 55° C., with the water reaching not more than half-way up the column of fluid in the tubes.

Emulsions of dysentery bacilli agglutinate much less rapidly than those of the enteric group; in consequence the tubes should be kept in the water-bath for at least four and a half hours, some workers even allowing them to remain 18 to 24 hours before the final readings are made.

If a water-bath at 55° C. is not available, the tubes may be placed in the incubator at 37° C. for not less than four and a half hours, and then be kept at room temperature for a further period of 16 or 20 hours before the final readings are taken.

By means of agglutination tests the diagnosis of *B. dysenteriae* (Shiga) is both simple and satisfactory, as all the bacilli classed under this name belong to a single serological group and are agglutinated approximately to titre by any Shiga serum.

(c) *Serological Races of Flexner's Bacillus.*

In the case of *B. dysenteriae* (Flexner) this is not so, as there are undoubtedly several serological races, attention having been drawn to this fact by W. Kruse[1] in 1907. Recent researches by Gettings, Murray, and Andrewes and Inman have served to confirm the fact that the Flexner-Y group can be split up by means of agglutination and absorption tests into a number of sub-groups. Andrewes and Inman, whose work is the most complete and was carried out with strains of dysentery bacilli collected from various sources, employed Dreyer's technique in their agglutination and absorption tests. Their results were summed up in the report of a sub-committee appointed by the War Office to consider certain questions in connexion with bacillary dysentery in the following words:

'The results suggested the existence of at least four distinct antigenic components in the group, provisionally lettered V, W, X, and Z.[2] Any one of those four may so preponderate in different strains as to impart a distinct serological facies. The corresponding agglutinins are not mutually absorbed except to a slight degree, and no one of the four can be more than partially absorbed by heavy doses of the other three combined. In addition to the four serological races thus defined, there remains the true Y group of Hiss and Russell, which presents differences from the others in agglutinability and agglutinogenic capacity, but in which there is not, so far, sufficient evidence of any fifth antigenic component, inasmuch as its agglutinin can be very largely absorbed by a combination of V, W, X, and Z strains.

'Further, two minor groups have been worked out in which two antigenic components were more evenly balanced, so that the serological behaviour was intermediate in character: one was a variety of the V race containing a large Z element and the other was intermediate between W and X.

'These conceptions were applied to an extended series of upwards of a hundred Flexner-Y strains from all parts of the world, but chiefly from France. Tested against half a dozen selected sera embracing all the types discovered, it was found that all except five or six strains could readily be referred to one or other of the seven races above described. Five sera, V, W, X, Y, Z, were required for complete diagnosis by agglutination.

[1] *Ztschr. f. Hyg. u. Infektionskrankh.* 1907, 57, 417.

[2] V is identified with the original Flexner strain; W with the Y of the Pasteur Institute and Oxford laboratories; X and Z have not been previously defined.

'The groups indicated by Andrewes and Inman corresponded in every particular with those elucidated by Murray; save that Murray had placed Z and X together as one type. Three of the groups, V, Z, and W, were identical with those established by Gettings in Asylum dysentery. No precise correlation with Kruse's races of "pseudo-dysentery" is at present possible.

'It was not maintained by Andrewes and Inman that there was no overlapping in their groups, *qua* agglutination; this could not be expected in view of the complex antigenic structure prevailing in the Flexner-Y species. Their results, nevertheless, appeared somewhat sharper than those of Gettings and Murray, possibly because they employed Dreyer's technique for agglutination. It was, however, contended that the groups were sufficiently distinct to render their recognition important in laboratory work on dysentery, in prophylactic vaccines, and probably in serotherapy. It has not yet been determined what degree of protection they are able or unable mutually to exert. Observations on this point are much required. The groups defined by Andrewes and Inman appear to embrace all the types of Flexner-Y bacilli met with during the present war, but the grouping is not put forward as a final one.

'The Committee, having considered the evidence as to serological grouping contained in the work of Gettings, Murray, and Andrewes and Inman, were of opinion that the existence of serological types in the Flexner Y-species, even though these might not be defined with absolute sharpness, was sufficiently established. It was agreed that, for the full recognition of these types by agglutination, five sera were desirable, though for practical purposes the four sera advocated by Murray were sufficient if it were not desired to distinguish between the Z and X races of Andrewes and Inman. It was agreed also that five agglutinable emulsions were probably requisite for complete sero-diagnosis in the Flexner-Y group of dysenteries, and that five types must be taken into account in serotherapy and in the preparation of vaccines.

'Of the five main types, Y can certainly be identified with the historical strain of Hiss and Russell, and V, with some probability, with the original Flexner strain, certainly with what is always termed Flexner's bacillus in European laboratories. W is identical with the so-called Y of the Pasteur Institute and the Oxford laboratories, while Z and X have not previously been defined unless they are included in Kruse's races. With regard to the so-called 'Strong' type, no reliable data can be obtained; it was chiefly based on the fermentation of saccharose, and no authentic strain is available for serological study; the term had best be abandoned.[1] The species, in the opinion of the Committee, should retain the name Flexner's bacillus (or *B. Flexneri*), and its hitherto defined races may be known as V, W, X, Y, Z.

'Certain practical results follow the recognition of the serological sub-grouping of the Flexner-Y species, but a distinction must be drawn between methods which will be of practical value for routine

[1] A suggestion with which Strong himself is understood to be in agreement.

use and those more elaborate and lengthy ones which may be needed for special purposes of research.

'For the routine diagnosis of members of the Flexner-Y group as such, the Committee recommend a polyvalent serum prepared by pooling five monovalent sera corresponding to the V, W, X, Y, and Z groups. This single serum will offer a simplification of the existing routine practice, for, together with a Shiga serum, nothing else will be needful for the serological diagnosis of the two chief species concerned in epidemic dysentery. For those workers who desire to study their strains more closely, as may often be needful in epidemiological investigations, the five monovalent sera can be issued separately. . . . It is probable that a considerable increase in positive results would attend the introduction of five agglutinable emulsions of the Flexner-Y species for sero-diagnosis.'

Although Shiga's and Flexner's bacilli are undoubtedly the most important organisms concerned in epidemic bacillary dysentery, there are others, occurring with lesser frequency, which have some claim to be regarded as concerned in the causation of the disease. Three such types may be mentioned.

(d) *Schmitz' Bacillus.*[1]

This organism resembles Shiga's bacillus in all its morphological and cultural characters, save that it freely produces indol—a property which at once serves to distinguish it. It always gives strong acid agglutination with Michaelis's test. It has no serological relations with Shiga's bacillus, and different strains exhibit a good deal of serological variation amongst themselves. Some observers report it as exhibiting a high degree of toxicity for animals, others as almost non-toxic. This organism was frequently met with on the Western Front during the war, but not in such fashion as to suggest any definite relation with epidemic dysentery. K. E. F. Schmitz, however, ascribes to it a causal relation in an epidemic of dysentery amongst Roumanian prisoners, and observers in the Balkans were of opinion that it was a cause of the disease. There was, moreover, an outbreak in Italy which appeared due to the same organism. It is apparently identical with that for which Andrewes [2] proposed the name *B. ambiguus*, in ignorance of the fact that Schmitz had already described it as *B. dysenteriae* (Schmitz).

(e) *Gettings' Bacillus.*

This bacillus was isolated by Gettings from several cases in a small outbreak of Asylum dysentery at Wakefield. He regarded it as the causal agent, and it is decidedly pathogenic for the rabbit. In its cultural characters it is identical with Flexner's bacillus, but

[1] The remarkable mutations which cultures of Schmitz' bacillus subsequently underwent in Germany throw considerable doubt upon the nature of this organism. See *Centralbl. f. Bakteriol.* (&c.), Abt. I, 1919, Orig. 83, 1 and 108.

[2] *Lancet* 1918, i. 560.

differs from that organism in giving strong acid agglutination, and in the fact that it has no serological relationship with Flexner's bacillus. Though freely agglutinable with its own serum, it refuses to agglutinate with the sera of any of the five chief Flexner types. It is probably to be regarded as a cause of dysentery, but of a different species from Flexner's bacillus.

(f) *Lactose Fermenting Types allied to Flexner's Bacillus.*

There are several organisms allied to Flexner's bacillus, and agreeing with it in general cultural characters, but forming acid with lactose. Some ferment this sugar early, others may take two or three weeks to form acid, and these latter types are peculiarly liable to be confounded with Flexner's bacillus. There is thus probably more than one species in this group, and this opinion is strengthened by the fact that they vary in their reaction to acid agglutination: most are strongly agglutinated by acids, but some not at all. Serologically they are widely separated from Flexner's bacillus, and this in two ways. Their velocity of agglutination is very much less and they require 20 to 24 hours at 55° C. for the reaction, whereas at that temperature Flexner's bacillus has almost reached its maximum of agglutination in 6 hours, though occasional exceptions to this rule have been observed in the W race of Flexner. In the second place even high titre sera prepared with a lactose-fermenting strain fail to agglutinate any of the Flexner races, and for the most part Flexner sera will hardly agglutinate the lactose fermenters, though such agglutination is occasionally observed to a small degree. These facts appear to separate the lactose fermenters as a group from Flexner's species.

It must be remembered that many dysentery bacilli, including not only Flexner's but also Shiga's bacillus, possess a very slight power of forming acid from lactose, not easily recognized in ordinary media, and that observations are recorded in which lactose fermenting mutants have apparently been thrown off from colonies of ordinary Flexner bacilli.

Nevertheless there is a certain amount of evidence to support the belief that they may be concerned in the causation of dysentery. Their pathogenicity for the rabbit is undeniable, and fully as great as that of Flexner's bacillus, showing similar pathological lesions. These organisms are not infrequently recovered from the stools of dysentery cases, and many observers, including Kruse, have not hesitated to include them amongst the dysentery group. The subject is one requiring further study.

The last three types of organisms do not complete the list of bacteria which have been claimed as causal agents in dysentery. There is, for instance, the 'Saïgon type' of the Pasteur Institute, found also in Gallipoli, and several others. But in all these cases further observation is needed before any conclusion can be reached.

Better established is the type of dysentery bacillus described by Sonne,[1] in 1914, as not uncommon in Denmark. It forms Sonne's Group III, and has also been found with some frequency in Norway by Thjötta [2] but has not yet been recognized in England:

it has received no specific name. An example of this strain has come into the hands of the Committee, and its investigation has confirmed the statements made by the Scandinavian observers. It is a late lactose fermenter and a late saccharose fermenter; glucose and mannitol are readily fermented, but dulcitol is untouched. It is only weakly agglutinated by any Flexner serum, but one serum of the X type, out of three tested, agglutinated it to 30 per cent. of full titre. Its own serum fails to agglutinate any Flexner type. It has thus little or no serological relation with Flexner's bacillus, and exhibits marked acid agglutination with Michaelis' solutions when a trace of normal serum is added to the mixtures. For these reasons it cannot be included in the same species as Flexner's bacillus, and it is equally unconformable with Gettings' bacillus. It should probably rank as a distinct species. According to Thjötta it often causes a mild diarrhoea rather than true dysentery, but he was readily able to demonstrate specific antibodies in the sera of the patients.

References.

[1] Sonne, Carl, *Giftfattige Dysenteribaciller*, Copenhagen, 1914.
[2] Thjötta, Th., *J. Bact.*, 1919, 4, 355.

(3) Acute Food Poisoning.

In this section only that common type of gastro-enteritis will be considered which is due to infection with members of the Salmonella group of bacteria: other forms of food poisoning are outside the scope of the present report. The bacilli concerned are Gärtner's *B. enteritidis*, *B. paratyphosus B*, *B. suipestifer*, and the so-called 'Aertrycke' bacilli. These organisms are alike in their cultural characters, and can only be distinguished by serological means: considerable doubt exists as to whether they should be regarded as distinct species or merely serological races of one species. The latter is perhaps the more probable view, but Gärtner's bacillus is serologically so distinct from the remaining three that it may possibly rank as a separate species. German writers group together all cases of food poisoning due to the paratyphoid group as 'gastro-enteritis paratyphosa'. In this country it is recognized that the strains concerned in food poisoning nearly always belong to the 'Aertrycke' group and are not serologically identical with the organism found in paratyphoid B fever. The term *B. suipestifer* has been loosely used for several races from swine fever in pigs: Bainbridge identified the original Aertrycke strain with *B. suipestifer*, but this identification is doubtfully of general application. The strains which now pass current in this country as 'Aertrycke' bacilli belong to at least two serological races known as the 'Mutton' and 'Newport' types. They are more closely allied to *B. paratyphosus B.* than to the common type of *B. suipestifer*, but they can only be distinguished from each other and from *B. paratyphosus B.* by the absorption test. The commonest strain concerned in food poisoning in England is the 'Mutton' type: the 'Newport' type and Gärtner's bacillus are less frequent, while

infection with the true *B. suipestifer* is almost unknown in this country, though outbreaks have occurred on the Continent.

The bacteriological diagnosis of acute food poisoning must be considered under two heads—(1) the recognition of the infecting bacillus in the persons affected, during life or after death, and (2) its recognition in the food which has set up the disease.

(1) *Diagnosis in the patient.* If cases can be examined in the acute stage, little difficulty usually presents itself, as the bacilli are then commonly abundant in the loose stools. The examination is carried out in the same manner as in cases of typhoid or paratyphoid fever, using the same differential media, and, if needful, the brilliant green enrichment method. Blood culture should not be neglected at this stage, and cultivations should also be made from the urine. If the acute symptoms have passed off there is still a reasonable prospect of recovering the bacillus from the stools or perhaps from the urine, but after a week or two the bacilli are not easily found.

The agglutinating properties of the serum may be tested at these later stages, using emulsions of the known bacteria concerned in food poisoning. This method is less uniformly successful than in the enteric group of diseases, the titre exhibited by the serum being usually lower, and the agglutinins disappearing more quickly.

In fatal cases the infecting organism is readily recovered from the heart's blood, spleen, liver, and other viscera.

(2) *Diagnosis in articles of food.* The common vehicle of infection is the flesh of pigs and cattle, very rarely of sheep,* and cured or preserved foods are especially liable to be infected. Ham, brawn, and meat pies have often been the source of food poisoning. Very frequently the bacteriologist is at a grave disadvantage because some days may have elapsed before his services are called in, and the uneaten remains of the food may have been thrown away. But sometimes in such cases the tins or other vessels which have contained the food may be available, even if they have to be retrieved from the dust heap, and may be examined with success.

There are two ways of examining food for members of the Salmonella group. The first is by ordinary culture, the material being plated out in the same manner as faeces are treated. The second is by feeding experiments on animals, mice being usually chosen, as they are extremely susceptible to these infections, but the method is not free from objection, as rodents are often carriers of the Salmonella group.

The Differential Diagnosis of members of the Salmonella Group. The cultural reactions of all the food poisoning bacilli of the Salmonella group are similar: those from human sources ferment glucose, mannitol, and dulcitol with formation of gas: all are negative in lactose and saccharose: none form indol and all produce alkali in litmus milk: all are actively motile.

When an organism has been thus established as a member of the group, the next step is to test its agglutinability with a Gärtner serum, and a Paratyphoid B serum. High agglutinability with

* For an instance of an extensive epidemic due to mutton, see Bruns u. Gasters, *Zeitschr. f. Hyg u. Infektionskr.* 1920, 90, 263.

the former proves it to be Gärtner's bacillus and no further test is needed. High agglutinability with the latter proves it to be an 'Aertrycke' or *B. paratyphosus B.* but gives no indication which it is. Low agglutinability with the Paratyphoid B serum, to ¼ titre or so, leaves the matter open and in such a case the bacillus should be tested with a *B. suipestifer* serum of standard strain.

The usual decision with which the bacteriologist is confronted lies between three organisms, the 'Newport' and 'Mutton' types of 'Aertrycke' and *B. paratyphosus B*, and the question can only be settled by absorption tests (see p. 137). For this purpose sera of all three types are required. If, on saturating Paratyphoid B serum with a heavy dose of the suspected bacillus, all the Paratyphoid B. agglutinin is removed, together with the agglutinin for the strain which is being tested, the organism is *B. paratyphosus B.* This will rarely be the case in food poisoning: it will probably be found that while all the agglutinin for the strain which is being tested is removed, that for *B. paratyphosus B* is but slightly reduced. This shows the organism to be of 'Aertrycke' type, and similar absorption tests with the 'Newport' and 'Mutton' sera will decide which.

(4) Other Bacteria causing or associated with Acute Intestinal Diseases.

(a) *Morgan's Bacillus.**

This organism, which resembles the other members of the coli group in its morphology, staining reactions, growth on agar, and inability to liquefy gelatine, is motile and has the following fermentation reactions: glucose is fermented with the production of acid and gas, but lactose, saccharose, dulcitol, and mannitol are unaffected. Indol is formed in suitable peptone media. The methods employed in isolating it from the faeces are similar to those described in the case of the enteric group of bacilli and it has been found that the use of suitable concentrations of brilliant green (see p. 39) renders the isolation more certain.

Much evidence has been collected implicating Morgan's bacillus as a cause of infantile diarrhoea. For instance, in a selected group of severe cases it was isolated from 63 per cent. by Morgan[1, 2] and his fellow workers.

In other epidemics, however, this high percentage was not maintained. It has never been claimed that this organism was the sole cause of infantile diarrhoea.

Agglutination tests were positive in some convalescents although not in high dilutions. Certain strains of the bacillus, when administered by the mouth to young rats and rabbits or adult monkeys produced violent diarrhoea and death. Autolysed cultures injected into the ear-vein of rabbits in medium-sized doses produced death with marked lesions of the intestine.

In fatal cases of infantile diarrhoea the organism was isolated

* The term, Morgan's bacillus, is here used for the organism originally known as Morgan's bacillus No. 1.

from the following situations at post-mortem examinations. Sixteen out of 33 cases gave positive findings.

Jejunum	1
Colon	13
Spleen and mesenteric glands	3
Liver and bile	2
Lung	1
Kidneys and urine	3
Heart blood	3

Although found during an epidemic in the faeces of a certain proportion of children not having diarrhoea, its presence is very uncommon in non-epidemic periods.

These results have been confirmed and amplified by the work of Lewis [3] and of Alexander [4] during the epidemic of 1911 at Birmingham and Liverpool. In their published papers and also in that of Graham-Smith [5] valuable data are adduced regarding the varieties of non-lactose-fermenting bacilli found in the stools of healthy children and in those suffering from diarrhoea and also regarding those isolated from milk and flies.

As a cause of enteritis in adults the evidence is not so conclusive, but it was found both by certain French [6] and English [7] workers to be very commonly present in the stools of patients who had contracted diarrhoea in Gallipoli.

Recent researches [8] have confirmed the earlier views that most probably there are a number of serological races of Morgan's bacillus.

References.

[1] Morgan, *Brit. M. J.*, 1906, i, 908 ; 1907, ii, 16.
[2] Morgan and Ledingham, *Proc. Roy. Soc. Med.*, 1909, 2, Part II, 133.
[3] Lewis, *Loc. Gov. Board Report* 1911–12, Supplement 265.
[4] Alexander, *ibid.* 288.
[5] Graham-Smith, *ibid.* 304.
[6] Tribondeau and Fichet, *Ann. de l'Inst. Pasteur*, 1916, 30, 357.
[7] Douglas and Colebrook, *Medical Research Committee, Special Report Series*, No. 6, 1917, 46.
[8] Kligler, I. J. *J. Exper. M.*, 1919, 29, 531.

(b) *B. pyocyaneus.*

This organism, the isolation and recognition of which are usually easy on account of the rapid production of the characteristic pigment, is occasionally found in small numbers in the faeces of healthy individuals. At times, however, it seems the causal agent in certain cases of enteritis and dysentery. This subject has been fully reviewed in a paper by Justi [1], who concludes that the presence of *B. pyocyaneus* in the intestine is usually harmless.

Adami appears to have been the first to draw attention to this form of enteritis. In Justi's opinion the cases reported by Baker, Wollstein [2], and Baginski [3] were undoubtedly infections due to *B. pyocyaneus*. All these cases were in children, the symptoms varying from dyspepsia, great weakness, and haemorrhages into the skin, to those of severe dysentery.

Calmette [4] records cases from the tropics occurring in adults.

Lartigau [5] reports an epidemic of dysentery (15 cases, adults and children) occurring near New York in which *B. pyocyaneus* was isolated in almost pure culture from the stools and also from the well water responsible for the epidemic.

The findings in fatal cases show that the lower part of the ileum is usually affected, but at times the colon also participates in the inflammatory changes; Wollstein reports actual ulceration; Marchand haemorrhagic thickening of the small and large intestines with necrosis.

The committee would, however, point out that it is a common experience in cases of dysentery or cholera that plates made from the faeces often show numerous colonies of *B. pyocyaneus*; these may be so abundant as completely to mask the causal organisms of these conditions.

In nature, *B. pyocyaneus* appears to be widely distributed, occurring in sewage, on vegetables, and in water. The latter seems to be commonly infected, as in 2,000 water examinations made in France it was found in 5 per cent. Bonjean [6] found it in water from the Holy Well at Mecca. The organism is pathogenic for mice, rabbits, and guinea-pigs. The extraction of pyocyanin renders its identification certain. It does not ferment lactose and the colonies, on a MacConkey plate, may in 24 hours simulate those of other pathogenic species, though usually more irregular in outline.

References.

[1] Justi, *Arch. f. Schiffs- u. Tropenhyg.*, 1915, 19, 458.
[2] Wollstein, *Arch. Pediat.*, 1898, 14, 760.
[3] Baginski, *Centralbl. f. Bakteriol.* [&c.], 1897, 22, 220; 1908, 47, 427.
[4] Calmette, *Arch. de méd. nav.*, 1892, 57, 277.
[5] Lartigau, *J. Exper. Med.*, 1898, 3, 595.
[6] Bonyean, *Ann. d'Hyg.*, 1899, 3. Ser., 43, 28.

(c) *B. proteus.*

B. proteus vulgaris and its near allies are easily distinguished from members of the colon group of bacilli by their power of rapidly liquefying gelatine.

The use of fermentation tests in the identification of this bacillus is unsatisfactory, partly owing to the property it often possesses of bleaching most of the indicators employed, and partly owing to its power of breaking down nitrogenous materials into alkaline substances which tend to neutralize any acid produced by the fermentation of the sugar. Under ordinary conditions of growth it does not appear to ferment either lactose or mannitol. Milk is clotted and peptonized. The organism can be easily recognized by other characters, such as the formation of a spreading opalescent film over the surface of the medium in ordinary agar cultures—a property making its isolation very simple—the production of a high degree of alkalinity due to the formation of ammonia from urea when grown in urine at 37° C. for a few hours (4 to 6), and its rapid liquefaction of gelatine. (See a review by Bulloch of recent work in *Medical Science* [1] and the exhaustive articles by Bengtson [2] and Wenner and Rettger [3].)

Attention was first drawn to this organism as a cause of infantile diarrhoea by Metchnikoff[4], who isolated it from 204 out of 218 cases of this disease between 1909 and 1913. He was also able to produce violent intestinal symptoms resembling cholera in the chimpanzee and in breast-fed rabbits by feeding experiments. His conclusion was that it played a very important rôle in the production of this infantile intestinal disease.

Bertrand working in London and Tsiklinsky[5] and Horowitch[6] in Russia confirmed these results. L. Bahr[7], however, working in Denmark examined the stools of 117 typical cases of summer diarrhoea and found *B. proteus* on only twelve occasions. He concludes that this organism plays no important part in the etiology of this disease in that country. Gildemeister and Baerthlein[8] working in Germany concluded that although it is not definitely proved that *B. proteus* is a cause of infantile diarrhoea its importance cannot be disproved. With this non-committal attitude the Committee are in agreement. There seems no sufficient evidence that *B. proteus* is a primary cause of enteritis whatever may be its importance as a secondary infective agent.

Organisms included under the name of *B. proteus* will most probably be found to belong to several different types; Van Loghem[9] described a race which did not form indol,[10] an observation confirmed by Groot[11] and Baudet[12]. The so-called *Proteus Zenkeri*, a non-liquefying species, probably belongs to a different group and is not known to possess any pathogenic significance.

Agglutination and absorption tests have shown that there are several serological races, and although sera produced by means of certain strains agglutinated to titre all the varieties tested, those prepared from other strains only agglutinated to titre a small proportion. These results would appear to indicate the presence of an antigenic structure which varies widely in different strains.

References.

1 Bulloch, *Med. Science*, 1919, 1, 213.
2 Bengtson, *J. Infect. Dis.*, 1919, 24, 428.
3 Wenner and Rettger, *J. Bact.*, 1919, 4, 331.
4 Metchnikoff, *Ann. de l'Inst. Pasteur*, 1914, 28, 99.
5 Tsiklinsky, *ibid.*, 1917, 31, 517.
6 Horowitch, *ibid.*, 1916, 30, 307.
7 Bahr, *Centrabl. f. Bakteriol.* [&c.], 1912, Orig. 66, 335 and 365.
8 Gildemeister and Baerthlein, *Centralbl. f. Bakteriol.* [&c.], 1913, Ref. 57, 263.
9 Van Loghem, *Fol. microbiol.*, 1915, 3, 8.
10 ,, ,, *Centralbl. f. Bakteriol.* [&c.], 1919, Orig. 82, 449.
11 Groot, *Ann. de l'Inst. Pasteur*, 1918, 32, 299.
12 Baudet, *Fol. microbiol.*, 1913, 2, 261.

5. Cholera.

The diagnosis of cholera and the identification of 'carriers' of the cholera vibrio can only be made with certainty by bacteriological methods. As the vibrio of cholera rarely passes through the intestinal wall into the lymph and blood-streams, even in the acute stage of the disease, it is in the intestinal contents that search has to be made for this organism; examinations of the blood and urine by cultural methods are, with very few exceptions, useless.

* Wolf examined thirteen strains isolated from wounds, none of which formed indol. See Wolf, *J. Path. and Bacteriol.*, 1919, 22, 289.

In the case of 'carriers' the focus of propagation is often the gall bladder, but as it is difficult without complicated measures to obtain a sample of bile it is by examination of the faeces that the presence of the vibrio is ascertained.

(a) *Principles governing the Isolation of* V. cholerae.

The principles governing the isolation of the cholera vibrio from faeces are the same as those employed in connexion with the members of the enteric group of diseases (see p. 32) although of necessity, as the properties of the *Vibrio cholerae* differ notably from those of that group, the methods and media employed differ correspondingly. Reliance is placed on the employment of (i) enrichment media and (ii) differential media. Before describing the methods used in isolating the cholera vibrio it will be necessary to describe the principles of both these types of media.

(i) *Enrichment media.* These consist of fluid media which are employed to increase the relative number of the cholera vibrio in a given sample. One of the most important points in all media used in the cultivation of vibrios—and this refers equally to solid as to liquid media—is the degree of alkalinity. *V. cholerae* and many other vibrios resembling it will proliferate in media so alkaline that *B. coli communis* and many other faecal bacilli fail to develop.

Making use of this property and of the extremely rapid proliferation of vibrios in a simple peptone solution, the commonest enrichment medium employed is peptone water which has been rendered very distinctly alkaline (P_H8·0 to P_H9·0). In practice this solution has proved so simple, so easily prepared, and so efficient, that although many other more complicated enrichment media have, from time to time, been advocated, it is still by far the most commonly employed, and it is doubtful if any other is more efficient. One, however, gives at least equally good results and may at times be of distinct advantage, as it appears that in it the cholera vibrios continue to increase in relative numbers for a longer period than is the case when peptone water is employed. This is Goldberger's alkaline egg medium, which consists of a mixture of the yolk and white of egg held in solution by sodium carbonate.

An important point as regards the action of these enrichment media is that not only do the cholera vibrios proliferate much more rapidly in them than any other faecal organism, but they also tend to form a surface film; hence it is frequently found that if a microscopical preparation is made, or plates are planted with material taken from the surface of these fluid media, the organisms found are practically all vibrios, yet samples taken from the depth of the medium may show a very large number of contaminating organisms. The practical aspect of these observations will be referred to when the methods used in the isolation of vibrios from the faeces are described.

(ii) *Differential media.* These media are employed in a solid form, usually in plate cultures. On a good differential medium colonies of *V. cholerae* rapidly develop, while the growth of most of the faecal bacteria is totally inhibited. The medium most widely employed is that introduced by Dieudonné. It consists of ordinary

neutral nutrient agar to which has been added, just before the plate is poured, a considerable proportion of a mixture of ox blood with normal caustic potash (see Appendix). The results obtained by its use are excellent when it is carefully employed. Dieudonné's medium has, however, one disadvantage, which is that the plates must be kept for a period of at least 24 hours after being poured before use.

Various modifications have been introduced to overcome this drawback. Pilon showed that the medium absorbed carbon dioxide from the air during the period of ripening, and if the plates were placed in an atmosphere rich in CO_2 they became suitable for implantation in a very much shorter period. This has been confirmed by one of us, who found the plates suitable for use after having been placed under a bell-jar containing a large proportion of CO_2 for one hour. Pilon also recommended that the ox blood should be mixed with sodium carbonate in place of normal caustic potash solution, and stated that this gave a medium of equal selective properties, which could be employed immediately. Hall stated that if the mixture of ox blood and caustic potash solution was kept for six or eight weeks in a flask plugged with cotton wool instead of an air-tight bung, the plates made with it could be used at once; this has also been confirmed by one of us.

J. Goldberger introduced a medium which he stated was equally selective with Dieudonné's: this is made by mixing nutrient agar with an alkaline egg mixture containing glucose. This medium gives more abundant growths of the cholera vibrio than Dieudonné's and can be used immediately, but from tests carried out by one of us it appears that its differential properties are distinctly inferior to those of Dieudonné's; further, the colonies of *V. cholerae* are less easily recognized on the opaque yellow background formed by this medium. The latter disadvantage is, however, easily overcome, without in any way detracting from the advantages, by substituting a suitable percentage of cane sugar for the glucose and adding a small amount of neutral red solution to the medium. When this is done the colonies of *V. cholerae* are very easily recognized, as, after the plates have been incubated for 24 hours, the centre of the colony becomes a deep red colour which shows very plainly against the yellow background of the medium. As most of the contaminating organisms have no action on cane sugar, the colonies they produce are quite colourless.

One other disadvantage of Goldberger's medium should be mentioned; this is that *B. proteus*, a bacillus present in some samples of faeces, develops as a continuous film over the whole surface of the plate, rendering the isolation of any other organisms impossible. On Dieudonné's medium *B. proteus*, when it develops, forms discrete colonies showing no tendency to spread.

(b) *Methods employed in Isolating* V. cholerae.

The details of the technique employed for the isolation of *Vibrio cholerae* vary slightly accordingly as the examination is carried out, (i) for the diagnosis of an acute case of cholera, (ii) for the identification of 'carriers'.

(i) *From the Faeces of an acute case of the Disease.* Having obtained a specimen of a rice-water stool, or, in a fatal case, material from the small intestine, a microscopical specimen is made from one of the epithelial flakes, which is picked out by means of a platinum loop, broken up on a slide, and stained with dilute carbol fuchsin. If large numbers of vibrios are seen in this, another epithelial flake is picked out, washed in several changes of salt solution, and then planted out on to the surface of a plate of Dieudonné's medium in a manner similar to that employed for planting out faeces when isolating the enteric group of bacilli (see p. 36). At the same time two or three other flakes are picked out, washed in salt solution, and planted into tubes of alkaline peptone water.

The plate is incubated for 18 to 24 hours, and if on examination large numbers of separate colonies of vibrios are found, the peptone-water cultures may be discarded. If, on the other hand, the plates are unsatisfactory, samples from the peptone-water tubes should be plated out. This is done by skimming the surface of the tube with a platinum loop so as to pick up any portion of bacterial scum that may have formed, as this frequently consists of an almost pure growth of vibrios. The loopful of material thus obtained is planted out on the surface of a plate of Dieudonné's medium.

If the case is one of cholera, numerous colonies of vibrios will certainly be obtained by this method after incubation of the plate for from 18 to 24 hours. Subcultures can then be made and with these the necessary tests are carried out to identify the culture as one of cholera.

If it is important to make a diagnosis as quickly as possible, plates should be made from the peptone-water tubes after these have been incubated at 37° C. for 6 and 12 hours as well as after an interval of 18 hours, as frequently the vibrios will have sufficiently outgrown any contaminating bacilli in the shorter period.

Some workers, before making the plate cultures from the peptone-water tubes, examine a microscopical specimen made from the bacterial film ; if this shows many contaminating bacilli a fresh peptone-water tube is planted, in the hope that after incubation for from 6 to 12 hours the number of vibrios will have relatively largely increased. This, however, does not always occur, as the contaminating bacilli may be capable of proliferating as rapidly as, or more so than, the *V. cholerae.*

If, instead of the typical rice-water stool, the specimen contains an admixture of faecal matter, it is usually better to discard the direct plate and to plant a loopful of the liquid stool into alkaline peptone water, and, after 6, 12, or 18 hours' incubation, to make plates from these enriching cultures on to a differential medium.

(ii) *From the Faeces of a Carrier.* As has been mentioned above, the focus of proliferation of these vibrios is frequently the gall bladder, but it is almost invariably in the faeces that the vibrio has to be sought, owing to the difficulty of obtaining samples of bile. Methods have been suggested, such as the administration of considerable doses of olive oil followed by drawing off the contents of the stomach into which the bile has regurgitated, also the passage

of a very fine rubber tube which is said to pass, after a variable period, into the duodenum, but these are beyond the limits of routine practice.

It is of the greatest importance that a mild purgative should be administered to the patient and that some of the liquid faeces thus obtained should be examined. Violent purgation of an individual who is temporarily harbouring the vibrios may, it should be remembered, induce an attack of cholera and should always be avoided during an epidemic.

A suitable sample of faeces having been obtained, a loopful of the fluid part should be planted into tubes of alkaline peptone water. These tubes are then treated in an exactly similar manner to those inoculated with material from an acute case.

If the vibrios are present in the faeces their recovery is a very much more simple and certain procedure than that of *B. dysenteriae* or members of the enteric group of bacilli from corresponding cases. Experiments have shown that the *V. cholerae* could be isolated with ease by the above methods when 25 cubic millimetres containing only 4 to 8 vibrios in a thick faecal emulsion were employed to inoculate the tubes of peptone water.

(c) *Identification of* V. cholerae.

The differentiation of the *V. cholerae* from other vibrios morphologically resembling it is made finally by serum tests: biochemical characters and the pathogenicity in respect to certain animals being employed only as preliminary indications.

The biochemical tests, &c., most usually employed are:

(i) Rapidity with which gelatine is liquefied.
(ii) Cholera red reaction.
(iii) Haemolysis of red blood corpuscles.
(iv) The pathogenicity in respect to certain animals.

(i) The *V. cholerae* liquefies gelatine somewhat more slowly than other vibrios.

(ii) The cholera-red reaction, which is said to depend on the fact that *V. cholerae* forms both nitrite and indol, consists in the production of a red colour on the addition of pure sulphuric acid to a 24-hour-old culture in broth or peptone water. It can always be obtained when the culture is one of *V. cholerae*, provided that the broth or peptone is suitable for its production. This reaction is obtained partially or completely with certain other vibrios.

(iii) Haemolytic test.[1] The *V. cholerae* does not produce a haemolysin, while most of the other vibrios isolated from water or faeces have marked haemolytic properties.

(iv) Pathogenicity. Recently isolated cultures of *V. cholerae* injected intraperitoneally into guinea-pigs in moderate doses produce a peritonitis which is fatal in from 15 to 24 hours. When injected subcutaneously, however, the animal remains unaffected. Subcutaneous inoculations into pigeons are also without effect.

Cultures of *V. Metchnikovi*, however, and those of certain vibrios which have been isolated from sporadic cases of a disease somewhat resembling cholera, when injected subcutaneously either into guinea-

[1] For the details of this test see Appendix, p. 90.

pigs, or better, pigeons, produce a septicaemia usually fatal in 24 hours.

These tests taken singly or collectively are not completely reliable.

Other differential features have been laid down by sundry observers, but of these none can be said to be constant. **The final diagnosis has always to be made by serum tests.**

The usual serum tests employed are:

(i) Agglutination tests.
(ii) Pfeiffer's reaction.

Of these, if the former gives positive results, the latter is not essential. Its value is as a test at once for pathogenicity and specificity. In an epidemic it is not necessary to employ this test with each culture. When a single suspicious case presents itself, it is of importance to apply it.

(i) *Agglutination tests.* These should be carried out by the methods which are described fully in Part II of this report.

The most suitable emulsion is a young broth or peptone-water culture diluted, if necessary, with normal salt solution,[1] to a suitable opacity. The titre of the serum should be known and the emulsion of the vibrio tested should show agglutination in a serum dilution corresponding to half or a quarter of the titre. In exceptional cases more than one monovalent serum may be required to establish the diagnosis.

(ii) *Pfeiffer's reaction.* For this test the following reagents, &c., are required:

1. The serum of an animal immunized with strains of known *V. cholerae.*
2. Three guinea-pigs, A, B, and C, each of about 200 grammes weight.
3. A 16 to 24-hour-old agar culture of the vibrio to be identified.
4. Pipettes, &c.

The test is carried out in the following manner: An emulsion of the vibrio to be identified is made by rubbing up a two-milligramme loopful of the culture in one cubic centimetre of broth; to this emulsion 0·001 cubic centimetre of the cholera immune serum is added and the mixture is then injected into the peritoneal cavity of guinea-pig A.

As a control, guinea-pig B is inoculated intraperitoneally with one cubic centimetre of an exactly similar emulsion of the vibrio to which has been added 0·001 cubic centimetre of normal serum. Guinea-pig C is inoculated with $\frac{1}{4}$ of a loopful (i.e. 0·5 mgrm.) of the culture emulsified in one cubic centimetre of broth.

Samples of the peritoneal fluid are withdrawn by means of capillary pipettes 20 and 60 minutes after the injections have been made and are examined either as hanging drops, or by dark ground illumination, under a cover-slip sealed with vaseline.

If the vibrio under investigation is a true *V. cholerae* the samples taken from guinea-pig A should show that the vibrios have lost all movement and have become granular, agglutinated into masses, and

[1] Preferably made with common salt, care being taken to filter the solution.

are undergoing bacteriolysis. Those from guinea-pigs B and C should show numerous normally shaped, actively motile vibrios. On the next day guinea-pig A should be quite normal, while B and C should be either dead or dying of acute peritonitis. If the vibrio under investigation is not a true *V. cholerae* the peritoneal exudate of guinea-pig A will show actively motile and otherwise normal vibrios.

The cholera immune serum employed in Pfeiffer's reaction must have such a titre that 0·0002 cubic centimetre injected into the peritoneal cavity of a 200 gramme guinea-pig causes complete bacteriolysis of 2 milligrammes of an 18-hour-old agar culture of *V. cholerae,* emulsified in one cubic centimetre of broth in 60 minutes and also protects the animal from a fatal peritonitis.

(d) *The Question of Serological Types of* V. cholerae.

It was formerly supposed that epidemic cholera could be caused by any one of a number of nearly related vibrios, all of which were grouped under the name *Vibrio cholerae.* Most authorities now agree that true epidemic cholera is due to a single species, *V. cholerae,* which, though occasionally varying in its cultural characters, is serologically a well-marked and homogeneous species. There is, however, a disease, generally occurring in small isolated outbreaks and having symptoms like those of cholera, though usually of milder type and followed by a lower case mortality. The vibrios recovered from such outbreaks, though having a general resemblance to *V. cholerae* in their morphology and cultural characters, are serologically quite distinct. These organisms have been termed *V. paracholerae,* and there appear to be several serological races.[1]

(e) *Paracholera and other Related Vibrios.*

The El Tor vibrios require special mention from the fact that an extensive literature has accumulated around them and complete unanimity has not yet been reached as to their exact position in relation to the true cholera vibrio. These El Tor strains (six in number) were recovered in 1905 by Gotschlich from the bodies of six Mecca pilgrims who died at El Tor in Arabia and who had presented no symptoms of cholera before death. Although no cases of cholera had occurred for some time in Arabia, these pilgrims had arrived from Asiatic Turkey and Russia in which countries cholera was then endemic. No doubt they were genuine cholera-carriers, though at that time the finding of cholera vibrios apart from cholera was regarded as a remarkable occurrence. By Gotschlich these strains were found to be agglutinated in high

[1] In order to confirm these facts, one of us tested nine strains of true cholera and two of paracholera, from widely varying sources, with sera prepared from one of the cholera and one of the paracholera strains. The nine cholera strains were all agglutinated to a practically equal degree by the true cholera serum, but not by the paracholera serum. The two paracholera strains were not agglutinated by the cholera serum. These results were completely confirmed by absorption experiments. Dreyer's technique with formalinized peptone-water cultures was used throughout: on repeating the experiments with saline suspensions of agar cultures, only confusing results were obtained. The evidence suggests that all true *Vibrio cholerae* strains belong to one serological race.

dilution by specific cholera serum and, further, the serum of animals immunized with the strains agglutinated not only the homologous organisms but also strains from typical cholera cases. The serological affinities of the El Tor strains to the true cholera vibrio have never been seriously disputed, but much controversy has arisen over the fact that the strains, unlike true cholera strains, produce a potent haemolysin and a quickly acting toxin for laboratory animals. Kraus and various co-workers, on the strength of this peculiarity shown by the El Tor vibrios, do not accept them as true cholera vibrios in spite of the serological affinities, and there can be no doubt that in this feature of haemotoxin production they do stand well apart from the generality of true cholera vibrios. On the other hand, it is held by another group of workers that the property of lysin production is a variable one not only for the El Tor vibrios but for genuine cholera vibrios also, and, consequently, that this property, though admittedly a very prominent one among the El Tor vibrios, does not suffice to exclude them from the group of *V. cholerae*.

V. paracholerae. While the cholera vibrio, as isolated from cholera cases wherever met with, forms a serologically homogeneous race, certain cholera-like vibrios, some of ancient lineage, others of more recent origin, require notice partly by reason of their etiological relationship with certain forms of choleraic disease in man, and partly because they may possibly represent intermediate phylogenetic links between the true cholera vibrios and those recovered from water, shell fish, and many other sources. These paracholera vibrios have no serological affinities with the true cholera vibrio, though, as Mackie and Storer have shown, they may form serological groups among themselves. The following may be mentioned :

V. Massawa (vel *Massaua*, *Massaouah*, *Massaua*, *Massuah*) was recovered in 1891 by Pasquale from the excreta of a case of cholera at Massawa in Eritrea. It was for long considered to be a true cholera vibrio. According to Kolle and Schürmann, however, it does not react to specific cholera serum. It is extremely toxic to guinea-pigs, rabbits, and pigeons, and Metschnikoff states that it preserves its virulence for a long time outside the body ; it is also said to possess four flagella.

V. Nasik was isolated in 1903 by Dr. Simond from a case of typical cholera at Nasik in India. Kraus, who examined this strain, found that it could not be regarded as a true cholera vibrio in view of the fact that a specific cholera serum of titre 1 in 20,000 agglutinated *V. Nasik* only up to 1 in 400. Moreover a serum prepared from *V. Nasik* did not agglutinate the cholera vibrio. This organism is highly haemolytic and has been the subject of much study by workers on bacterial haemotoxins.

V. paracholerae A and *B* (Mackie and Storer, 1916) were recovered by Mackie and Storer from the dejecta of six non-fatal, though clinically choleraic, cases which occurred in a British convalescent camp at Alexandria. In three of the cases the vibrios were very evident on microscopical examination of the discharges. The

serum of one patient agglutinated his own vibrio and also *V. cholerae* in dilutions up to 1 in 200 after 4 hours.

The strains were not agglutinated by specific cholera serum even in 1 in 50. Serologically in cross-agglutination experiments Mackie and Storer were able to separate the strains into two groups, one group containing four of the strains and the other two. The strains were fatal for rabbits by intravenous inoculation, but experiments on pigeons were negative. Haemolysis on blood agar was slow but quite evident after 36 hours.

V. Kegallensis (Castellani, 1913) was recovered by Castellani from cases in Ceylon 'clinically scarcely distinguishable' from cholera, with profuse serous diarrhoea, severe vomiting, cramps, &c. It was very abundant in the stools and the only species of vibrio present. A similar organism was recovered by him from a well near which a small epidemic of cholera-like cases occurred. The strain was not agglutinated by cholera serum in dilutions higher than 1 in 10 or 1 in 20, nor was cholera vibro agglutinated by the *V. Kegallensis* antiserum. There was no indol production and haemolysis was inconstant.

V. Gindha (vel *V. Gindha*, Pfeiffer, 1896) was recovered by Pasquale in 1891 from the water of a well at Gindha (near Massawa), near which, some months previously, a cholera epidemic had occurred. It has no serological affinities with the cholera vibrio.

More recently Chalmers and Waterfield have recovered from a case of paracholera at Port Sudan an organism which they have attempted to identify with *V. Gindha*. It was not agglutinated by cholera serum and gave a negative Pfeiffer reaction. Haemolytic action was well marked, indol was produced, and the cholera-red reaction was positive. One polar flagellum was present. The organism was not pathogenic for guinea-pigs or rabbits or pigeons.

Chalmers and Waterfield have made an elaborate classification of the paracholera and cholera-like vibrios, based largely on statements in the literature as to morphology, cultural and biochemical properties, &c. In the light of recently acquired knowledge of bacterial variation, however, such methods of classification are probably of diminished value, and what is required at the present time is a classification based on a simultaneous examination from all points of view, serological and otherwise, of a collected series of such paracholera strains.

Mackie and Storer's serological grouping of their paracholera strains was an advance in the right direction and it is worth noting that, during a cholera outbreak in Baghdad in 1917, an inagglutinable vibrio isolated by Major Gloster, I.M.S., from a mild case of suspected cholera was found by him to be agglutinated to titre by one of Mackie and Storer's paracholera sera sent from Egypt.

Particulars of strains at primary isolation and of the associated clinical picture are invaluable, and in connexion with paracholera strains generally the point should be remembered that inagglutinable cholera-like vibrios have been found in association with the true cholera vibrio in the same faecal specimen.

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V. The Diagnosis of Dysentery due to Animal Parasites.

(1) Amoebic Dysentery.

Introduction. The exact diagnosis of amoebic dysentery can only be made by the microscopical examination of the stools and by finding *Entamoeba histolytica* in them.

In order to carry out these examinations satisfactorily the observer must have an accurate knowledge of (i) the life-history of *Entamoeba histolytica*; (ii) the various stages of other entamoebae, e.g. *E. coli* and *E. nana*, that may be present in the stools and which may be confused with *E. histolytica*; (iii) other bodies, e.g. certain cells, cysts of other protozoa, &c, which may be mistaken for forms of *E. histolytica*.

(a) *Collection of Material, &c.*

This varies according to the kind of examination required:

(i) To diagnose acute, subacute, or chronic cases of dysentery.

(ii) To diagnose 'amoebic diarrhoea'.[1]

(iii) To ascertain if treatment has been successful or if the individual is a 'carrier'.

(i) The stage of *E. histolytica* that is found in acute, subacute, and chronic cases of dysentery is the active adult form, and as this stage tends to die and disintegrate very shortly after leaving the intestine it is of the **utmost importance that the examination should be made as soon as possible after the passage of the stool.**

It is difficult to give a definite statement as to an exact period within which the examination should be made, as individual cases show great variation. Occasionally within two hours of the passage of the stool most of the amoebae will have disappeared; in other cases living and active organisms can be found twelve and even eighteen hours after the collection of the specimen. The only safe course is to make the examination as soon as possible.

In the acute and subacute cases the stools consist largely of blood-stained mucus, and in this material the amoebae are usually found fairly uniformly distributed. If a sample has to be sent to the laboratory two or three spoonfuls should be placed in the faeces bottle described above. See p. 21.

If faecal matter is also present, a sample should be collected separately and placed in another receptacle.

It is also important that the bed-pan or other utensil into which the stool is passed should be free from antiseptics and dry, as even

[1] The term 'amoebic diarrhoea' is used to denote that type of disease in which blood-stained mucus is absent from the stools; the chief symptom is diarrhoea. The forms of *E. histolytica* found in these cases are usually the cystic and precystic, but a few of the active adult forms may also be present.

water rapidly kills the entamoebae. Care should be taken to avoid the admixture of urine with the stool.

In cases of chronic dysentery the amoebae are often much less evenly distributed throughout the mucus present in the motion. So marked is this in certain cases that many specimens may be examined without finding any amoebae, while others may show them even in very large numbers. It is therefore important in such cases to pick out from the motion any small blood-stained portion or slough that may be present, as it is usually in such material that the large numbers of amoebae are found. Faecal matter, if present, occasionally contains the precystic or cystic stage of *E. histolytica*.

(ii) When the examination is made for the diagnosis of 'amoebic diarrhoea', it is the precystic and cystic stages of *E. histolytica* that are especially sought for. The cystic stage is the one that can be most easily recognized, but the precystic is the most difficult. It is therefore of the utmost importance **that the administration of purgatives should be avoided,** as liquid stools contain chiefly the precystic forms.

The cystic stage of the amoeba is, moreover, much more resistant, so that there is not the same urgency in making the examination immediately after the passage of the stool; but if stained preparations are required, it must be remembered that in some cases nuclear degenerations and other cytological changes take place very soon after the cysts have been evacuated, so that the sooner the specimens are made and fixed the better.

In collecting the specimen a portion of the solid stool should, if possible, always be taken; and if this has to be sent to the laboratory it is best placed in the faeces bottle described above (see p. 21).

(iii) When the examination is made to ascertain if treatment has been successful or the individual is a 'carrier' it is the cystic stage that is most frequently sought for. In such cases it is therefore equally important to avoid the administration of purgatives and to take as a sample of the stool a portion of any solid faeces that may be available. If the specimen cannot be examined at once, it should be kept at room temperature and not be placed in the warm incubator. Specimens should never be taken during the administration of emetine.

(b) *General Remarks on the Preparation of the Microscopical Specimens.*

Microscopical specimens of two types are employed in the identification of the various stages of *E. histolytica*.

1. Temporary wet preparations.
2. Permanent preparations, fixed and stained.

The examination of all preparations should be systematic, and a mechanical stage is therefore essential for accurate work. An eye-piece micrometer is also required.

1. Temporary wet preparations are made by mixing or emulsifying a portion of the material in (*a*) normal saline, (*b*) iodine solution.

(*a*) In examining preparations mounted in normal salt solution,

the amoebae being colourless, the sub-stage diaphragm must be partially closed or the condenser racked down to render their outlines apparent. An artificial source of light is the best illuminant.

(*b*) The iodine solution employed is made by saturating a one per cent. watery solution of potassium iodide with iodine. This solution is especially employed when the cysts of *E. histolytica* are the objects of the search. The iodine solution acts partially as a fixative and partially as a stain, so that the internal structures are rendered more visible.

(c) *Description of the Adult Form of* E. histolytica *and its Differential Diagnosis from other Amoebae, &c.*

The active adult form of the amoeba is usually identified by the examination of unstained preparations. These are made by placing on a slide a small portion of the blood-stained mucus with a small quantity of salt solution and then making a thin film by dropping a cover-slip on to the mixture. The cover-slip may be ringed with vaseline to prevent evaporation. The specimen is best examined with a dry lens of $\frac{1}{6}''$ or $\frac{1}{8}''$ focal length, combined during the preliminary search with a very low ocular, which can be changed for one having a higher magnification on finding the organism. A warm stage is unnecessary for routine work.

The amoebae are recognized as highly refractive organisms which often show active movements. These movements consist of the extrusion of pseudopodia, which are often emitted with almost explosive suddenness as lobes of clear ectoplasm thrown out from the periphery of the organism. Occasionally under very favourable conditions the amoebae show a rapid progressive flowing movement over the surface of the slide. Usually more feeble locomotion occurs as the result of a large pseudopodium composed of ectoplasm being extruded, the endoplasm then following onwards into it. Several pseudopodia may be extruded simultaneously.

A typical adult *E. histolytica* has the following characteristics. When living but at rest the amoeba assumes a spherical form. The size of different individuals varies considerably, but they usually measure from 20 μ to 30 μ in diameter. The protoplasm is seen to consist of ectoplasm and endoplasm. The ectoplasm constitutes a well-marked outer layer of protoplasm which appears quite clear and structureless, but is so highly refractive that the outline of the organism is very sharply defined. When pseudopodia are thrown out from an amoeba which is not undergoing locomotion they are composed of ectoplasm only. The endoplasm is very finely and uniformly granular, which renders it markedly opaque when compared with the ectoplasm. Red blood cells, in various stages of erosion, are often seen embedded in it, but bacteria and other inclusions are not present in healthy specimens of *E. histolytica*. Vacuoles, resembling bubbles, often occur in degenerate individuals.

The nucleus cannot be made out clearly in a perfectly healthy amoeba, but in the earlier stages of degeneration it becomes easily visible. It is situated in the endoplasm and is almost always placed

eccentrically. Spherical in shape, it appears under the microscope as a finely beaded ring from 4 μ to 7 μ in diameter, occasionally with a very small central dot—the karyosome.

The most important characters for identifying this stage of *E. histolytica* in fresh unstained preparations are the active explosive movements, the frequent presence of red blood cells in the endoplasm, and the absence of other inclusions.

The adult stage of *E. histolytica* seen in unstained preparations has to be distinguished from

(i) *E. coli* and other amoebae.

(ii) Other bodies, such as cells, found in dysenteric stools.

(i) The active forms of *Entamoeba coli* differ from those of *E. histolytica* in the following points. The ectoplasm is much less conspicuous. The endoplasm is more granular and always contains many inclusions such as bacteria, yeasts, starch grains, and other vegetable remains. Cysts of other protozoa and *Blastocystis* are also sometimes seen, but red blood cells are never present. Vacuoles are common and are often somewhat spindle-shaped, giving the impression of dilated cracks in the protoplasm. The nucleus is conspicuous and can be made out as a beaded ring even in undegenerate forms. The karyosome when visible almost always appears as an eccentric granule or group of granules.

Movement is typically very slow or absent in *E. coli* and consists usually in a slight change of shape without locomotion. The pseudopodia show no sharp line between their ectoplasm and endoplasm.

The other species of amoebae occurring in the human bowel are not easily mistaken for the adult forms of *E. histolytica* as they are all of smaller size (less than 20 μ).

(ii) The other bodies that may be mistaken for *E. histolytica* in unstained preparations made from dysenteric stools are chiefly

(1) Degenerate amoeboid forms of *Trichomonas*.

(2) Cells derived from the host:

- (*a*) Endothelial.
- (*b*) Epithelial.
- (*c*) Mucous cells.

(1) The degenerate amoeboid forms of the flagellate *Trichomonas* which have lost their flagella may be distinguished from *E. histolytica* by carefully watching the organism—which is usually much smaller (commonly 5 μ to 15 μ)—when it will be noticed that no progressive movements occur and that the pseudopodium extruded is finger-like and travels round a portion of the circumference and is then withdrawn into the body. Normal flagellates can also usually be found in the specimen.

(2) Cells derived from the host.

(*a*) Endothelial cells, common in bloody stools, and derived from the vessels of the inflamed area, are the most important, as they are large and often contain inclusions such as red blood cells, and may show slight changes in shape. Careful examination, however, fails to show any distinction between ectoplasm and endoplasm. The nucleus, which can be easily seen, is much smaller, but is not ring-like, the chromatin appearing evenly distributed. Lastly, the cells

are flattened and plate-like, as can often be demonstrated by gently tapping the cover-glass and so causing the cell to turn over.

(*b*) Squamous epithelial cells derived from the anal margin are to be detected on the surface of all solid stools and in liquid stools may be present throughout the whole mass. They show a superficial resemblance to amoebae in their general contour and in possessing a large ring-shaped nucleus; but the absence of all movements, the central position of the nucleus, their flat shape and freedom from red blood cells and other inclusions, serve to distinguish them.

(*c*) Goblet cells may also occasionally be mistaken for *E. histolytica*, but here again the absence of all movements and inclusions and their general appearance render their differentiation easy.

(d) *The Identification of the Precystic and Cystic Stages of* E. histolytica.

These stages of *E. histolytica* are found in (i) cases of amoebic diarrhoea, (ii) cases under treatment, and (iii) carriers.

Two preparations should always be made in the examination of these cases, one being mounted in normal salt solution and the other in iodine solution. These specimens are made by evenly emulsifying on a slide a small portion of faeces in either solution, and covering it with a cover-slip.

The examination should be made with a dry lens such as a $\frac{1}{6}''$ or $\frac{1}{8}''$ objective, a low ocular being used in the preliminary search. For diagnostic purposes an immersion lens may be essential for making out some of the finer details.

The precystic stage of *E. histolytica* is much smaller than the active adult stage—the size differing in different races. Those races forming large cysts have a comparatively large precystic stage, while the races with smaller cysts have a correspondingly small precystic form. Precystic amoebae with diameters of 7 to 9 μ or 12 to 14 μ are the commonest, but the measurements may range from about 7 to 18 μ. In individual cases, however, usually only one race is present, so that all the amoebae in the precystic and cystic stages will either be large or small. Amoebae of different average sizes are present only when there is a double infection.

A sharp differentiation between ectoplasm and endoplasm cannot always be made out; movements are very sluggish or absent.

The protoplasm appears quite clear and contains no red blood cells or other inclusions. The nucleus is generally visible as a finely beaded ring. The karyosome is often visible as a more or less central granule or heap of granules. In size the nucleus is proportionally larger than in the adult stage, its diameter being equal to about $\frac{1}{4}$ of the diameter of the whole organism.

This stage of *E. histolytica* is the most difficult to identify, in fact it is often impossible to say whether a particular organism belongs to this species or to *E. coli*. It is only by the examination of a number of individuals and attention to the finer details that a diagnosis can be made. In this stage well-fixed and properly stained specimens may be of great use in forming an opinion. In practice it is often best

to continue the examination of the stools until typical cysts or adult forms appear in them and so confirm the diagnosis.

The cystic stage of *E. histolytica.*

(*a*) In salt solution the cysts appear as round slightly greenish bodies, their shape being typically spherical or oval. The cyst wall, as in all amoebic cysts found in human faeces, is uniformly thin, quite colourless, and shows a definite double contour. The size varies, according to the race, from about 6 to 18 μ in diameter. (These races have been mentioned in the description of the precystic stage.) The cysts of races forming small cysts commonly measure from 7 to 9 μ in diameter, while the dimensions of those belonging to races forming large cysts usually lie between 11 μ and 14 μ. Living and healthy cysts have finely granular protoplasm and often contain chromatoid bodies—bars or blocks of a highly refractile substance. The nuclei, one to four in number, are very inconspicuous in the healthy cyst, but in the early stages of degeneration they become more apparent. Cysts with more than four nuclei are practically never seen. Bubble-like vacuoles are usually present in degenerate cysts.

(*b*) In iodine preparations the whole cyst takes on a yellow colour, the nuclei become clearly visible and each appears as a slightly beaded ring with a tiny centrally placed dot—the karyosome (see Fig. 7, Pl. II). The size of the nucleus varies with the number present, the nuclei being largest in the uninucleate and smallest in the quadrinucleate cysts. In the former the nucleus roughly measures one-third of the diameter of the whole cyst, while in the latter each of the four nuclei measures only about one-sixth. The chromatoid bodies are less conspicuous than in cysts mounted in salt solution. Generally the cysts contain glycogen, which shows as a reddish-brown patch with an ill-defined outline. The size of these patches varies; sometimes they occupy nearly half the area of the cyst, and often more than one may be present.

(e) *Differential Diagnosis of Precystic and Cystic Stages of* E. histolytica *from other Amoebae, &c.*

The cystic and precystic stages of *E. histolytica* must be differentiated:

1. From the same stages of *E. coli.*
2. From the amoeboid and cystic stages of *Endolimax nana.*
3. From free and encysted forms of *Iodamoeba bütschlii* ('Iodine cysts' or 'I. cysts' (Wenyon)).
4. From cysts of other protozoa, and from other bodies that may be present in the faeces.

(1) The precystic stages of *E. coli* are almost identical with those of *E. histolytica. E. coli* is, however, usually slightly larger (14 μ to 18 μ in diameter) and the nucleus is more conspicuous, showing as a more coarsely beaded ring of chromatin granules with a larger and usually eccentrically placed karyosome. *Differentiation of these entamoebae in the precystic stage is extremely difficult and often impossible.*

Cysts of *E. coli* show very definite characters in which they differ from those of *E. histolytica*. In salt solution *E. coli* cysts show as white shining bodies, usually round but sometimes oval. They vary commonly from 15 μ to 20 μ in diameter. They may measure more or less than this, but are never below 10 μ and very rarely over 30 μ. The nuclei can usually be detected, under an oil immersion lens, in the living cyst. They vary from one to eight in number (very occasionally more than eight are found, e.g. 12 or 16) but eight is the number most usually present. Large chromatoid bodies are usually absent, but when present are typically in the form of highly refractive spicules or sheaves of spicules.

In iodine preparations the nuclei become very conspicuous and show as beaded rings, the beads of chromatin being coarser than those of *E. histolytica* and more irregular. One or more dots of chromatin can usually be seen within the nucleus. (See Fig. 12, Pl. II.)

In the uninucleate, binucleate, and quadrinucleate cysts, which are those most easily mistaken for cysts of *E. histolytica*, the nuclei are coarser and larger in proportion. In quadrinucleate cysts one or more nuclei will often be seen to be undergoing division, being drawn out into a spindle. Glycogen is generally present in these immature cysts, being most abundant at the binucleate stage, when it appears as a large mass often occupying the greater part of the cyst. These masses stain a deep mahogany brown with iodine and have a sharply defined edge. In cysts with eight nuclei the protoplasm stains uniformly pale yellow, no glycogen masses being present.

(2) The adult stage of *Endolimax nana* may be easily mistaken for the smaller precystic forms of *E. histolytica*. This amoeba is about 7 to 10 μ in diameter. No sharp distinction can be made out between the ectoplasm and endoplasm, and the movements usually become sluggish and cease shortly after leaving the body. The protoplasm is granular and contains as inclusions many bacteria, yeasts, &c., which lie in vacuoles. Red blood cells are never seen inside the organism. The nucleus of the living amoeba is always very inconspicuous.

Cysts of *E. nana* may be confused with cysts of the small races of *E. histolytica*; they are, however, usually oval, measuring about 8 to 9 μ in length. In salt solution they appear white, the protoplasm showing nothing but a few bright granules. The nuclei are invisible and chromatoids and vacuoles are absent.

In iodine preparations the nuclei—which are very small, and one, two, or four in number—appear very inconspicuous. (See Fig. 10, Pl. II.) Glycogen is sometimes present in immature cysts; when present it stains sometimes as a dark mass and sometimes as a diffuse pale patch. The bright granules can usually be seen against a pale yellowish background.

(3) The amoeboid forms of *Iodamoeba bütschlii* are not easily distinguishable when alive, or when mounted in iodine solution, from the precystic forms of *E. histolytica* and *E. coli* and the unencysted forms of *E. nana*. They are small amoebae of similar size, and resemble large individuals of *E. nana* or small individuals of *E. coli*. Outside the body they show only slight movements as

a rule, and very quickly degenerate. Usually they contain bacteria in their food vacuoles. To distinguish these amoebae with certainty it is generally necessary to make stained preparations, as their nuclei furnish the most certain characters for differential diagnosis (see p. 80).

The cysts of this amoeba are liable to be mistaken for those of *E. histolytica*. In saline preparations their shape is frequently irregular, but often oval, usually measuring 9 μ to 11 μ in diameter. They are very white and clear and contain a number of bright granules and a dull inclusion, of variable size, consisting of glycogen. The nucleus, which is always small and single, is invisible.

In iodine preparations the glycogen stains a dark mahogany colour and has a well-defined edge; occasionally it occurs as two or three masses and is sometimes absent. The nucleus is visible but inconspicuous; it is smaller than the nucleus of a uninucleate cyst of *E. histolytica* of corresponding size, and is signet-ring-shaped, having a large peripheral karyosome. (See Fig. 9, Pl. II.)

(4) Other bodies which may be mistaken for the cysts or precystic stage of *E. histolytica* are:

(i) Cysts of the flagellate Lamblia (*Giardia intestinalis*). These are always oval, 10 μ to 14 μ in length, clear, slightly yellowish or greenish in colour, and contain fine rod-like and crescentic inclusions. In iodine preparations two or four very small nuclei can be made out at one end of the cyst, and the filamentous inclusions become very conspicuous. (See Fig. 15, Pl. II.)

(ii) Cysts of the flagellate *Chilomastix mesnili* are lemon-shaped, having a small projection at one end; they are about 8 μ in length. The general appearance of the contents is clear with a few very bright granules. In iodine preparations the single nucleus can be seen; it is of large size and usually shaped like a signet ring. The outline of the mouth can also be made out, and appears as a sling extending from end to end. (See Fig. 13, Pl. II.)

(iii) *Blastocystis hominis*, a vegetable organism almost universally present in the human intestine, is frequently mistaken for a protozoal cyst. The living organism (Fig. 14, Pl. II) usually contains a large homogeneous mass of reserve material, around which the protoplasm is disposed in a very thin layer. In the protoplasm brightly refractile granules and one or more tiny nuclei are present. The organism varies from about 5 μ to over 20 μ in diameter, but such large specimens are uncommon. In iodine solution the protoplasm is coagulated, and consequently appears like a cyst wall enclosing the reserve mass, which stains a uniform yellowish colour and shows no internal structure. In soft stools specimens of various sizes are often found, and many individuals undergoing division—when they become hour-glass shaped and finally constricted into two—can usually be seen. (This organism is often incorrectly described as the cyst of *Trichomonas*.)

Polymorphonuclear leucocytes, large yeasts, starch grains, and oil drops are sometimes mistaken for cysts, but need only be mentioned here as possible sources of confusion or error.

It will be noted that, throughout this section, many methods which have from time to time been recommended as aids in the

discovery or recognition of the different stages of *E. histolytica* have not been mentioned. These omissions are intentional, as in the opinion of the Committee the faculty of recognizing, with certainty, the various stages of such organisms as amoebae can only be acquired by constant practice, together with an accurate knowledge of the minute anatomy of the different stages which make up the life-history of the organism. Once this is thoroughly mastered all such methods become superfluous, and their use in the hands of beginners often leads to inaccuracies. As instances of these methods may be mentioned the addition of various stains to the salt solution in which the faeces are emulsified, or the concentration of the cysts of amoebae by centrifuging after the addition of ether.

(f) *The Preparation of Stained and Permanent Specimens.*

As good permanent specimens are difficult to prepare and are only occasionally necessary in making a diagnosis, the method described will be a simple one. The preparation of these specimens is never easy and it is only by careful attention to details that satisfactory results can be obtained.

Amongst the most important of these are:

(i) The specimen should be prepared and fixed as soon as possible after the passage of the stool, especially when specimens of active amoebae are required; for, as has been pointed out above, the amoebae all rapidly die and disintegrate after leaving the intestine. Even when the specimens are made and fixed immediately after the stool has been passed, many individual amoebae will be found that show degeneration. There is not the same urgency in the case of specimens containing cysts, as even after some days a number of living cysts will usually be found in the stools.

(ii) In making these specimens, whatever stage of the parasite is sought for, the preparation must *never be allowed to dry.*

The following special reagents are required:

1. *Fixative.* A very convenient formula suitable for all stages of the parasites is the following: Saturated aqueous solution of mercury perchloride, 2 parts; absolute or 96 per cent. alcohol, 1 part. These are mixed together and to each 100 cubic centimetres of the mixture 5 cubic centimetres of glacial acetic acid are added. This solution keeps for a long period.

2. *Stain.* Haemalum solution, which is prepared as follows: One gramme of haematoxylin is dissolved in one litre of water, then 0·2 grm. of sodium iodate ($NaIO_3$) and 50 grm. of alum (potash) are added. When these salts are dissolved the stain is filtered. This stain has the advantages that it is easy of preparation, does not require to be kept before use, and is not liable to overstain.

Fixation. In preparing the specimens a small portion of the blood-stained mucus or an emulsion of faeces containing cysts is spread with a platinum loop over the surface of a cover-slip. As soon as the material has been spread out into a thin regular film the cover-slip is floated filmside downwards on the fixative, and placed in a watch-glass or small Petri dish. Care should be taken to avoid the formation of air bubbles between the film and the fluid,

and if the cover-glasses sink they should be turned filmside upwards, so as to expose the film properly to the fixative. A slide may be used instead of a cover-slip, but is less suitable as it is difficult to fix the film without making it run into ridges.

Fixation is complete in from 10 to 20 minutes, amoebae being more rapidly fixed than cysts, which should always be given the longer period.

Washing processes. The specimens must now be thoroughly washed, first in 50 per cent. or 70 per cent. alcohol, after which they are placed in 70 per cent. alcohol to which a few drops of iodine solution have been added. In this they are allowed to remain for 30 minutes, and are then placed in weaker alcohol (e. g. 35 per cent.) for a few minutes before being transferred to distilled water.

Staining. This is carried out by placing the cover-slips in the haemalum solution for 10 to 20 minutes, and in this process, as in that of fixation, the cysts require longer than the adult forms. The specimens will not usually be found overstained even if the staining process is prolonged considerably; but if overstaining occurs they may be decolorized in acid alcohol or weak alum solution.

After staining, the specimens are washed in tap-water until blue. No counter-stain is necessary, but watery eosin may be used, any excess of this stain being removed by prolonged washing in tap-water.

Dehydration, &c. This is preferably carried out in stages, the specimens being placed first in 35 per cent., then in 70 per cent., and lastly in absolute alcohol; from this they are placed in a mixture of equal parts of absolute alcohol and xylol, then in pure xylol and are then mounted in balsam. During these processes the greatest care must be taken that the specimen is never allowed to dry.

Careful and slow dehydration and clearing are necessary especially in the case of cysts, the walls of which show a great tendency to wrinkle, especially when being cleared, thus obscuring the details of the contents.

Many other methods, such as Heidenhain's iron haematoxylin or Weigert's iron haematoxylin, may be employed, but the above method brings out all the details necessary for the recognition of the different stages of *E. histolytica.*

(g) E. histolytica *in Preparations stained by the Method described and its Differential Diagnosis.*

In the adult stage the amoebae show as rounded pale lilac-blue bodies, the ectoplasm being distinguishable from the endoplasm by its transparency and lighter colour. Any red blood cells contained in the endoplasm can be made out as pale shadows. In size the stained organism is slightly smaller than the living, resting form, ordinary measurements being from 20 μ to 25 μ in diameter. The nucleus is seen lying eccentrically in the endoplasm, and although spherical in shape it appears as a finely beaded ring, 4 μ to 7 μ in diameter. These beads are composed of chromatin, and in the normal healthy organism are very regular in shape and very

small. Situated in the centre of the nucleus and surrounded by a clear halo is a minute chromatin karyosome usually less than 1 μ in diameter; no other beads of chromatin are present between the karyosome and the outer chromatin ring. (See Fig. 1, Pl. I.)

In degenerate organisms the cytoplasm may contain bubble-like vacuoles and bacteria. The chromatin ring of the nucleus becomes much less uniform and may be represented by a few irregular peripheral masses. Smaller fragments of chromatin may be seen scattered between the periphery and the central karyosome, which may be fragmented or even absent.

The precystic stage is very similar except that the organism is smaller and contains no red blood cells. (See Fig. 2, Pl. I.) The nucleus is slightly larger in proportion to the size of the amoeba, its diameter being about $\frac{1}{4}$ of the diameter of the whole organism. Both the peripheral chromatin granules and the karyosome are proportionately larger but have a similar arrangement, and chromatin is often present in the zone between them.

Degenerate forms show similar changes to those seen in the adult stages, and here again bacteria may be present in the endoplasm. These bacteria have invaded the amoebae and have not been taken up by the organism as in the case of adult forms of *E. coli* and *E. nana*. The precystic amoebae also occasionally contain chromatoid bodies like those seen in the cysts.

Cysts of *E. histolytica* when stained show as rounded bodies varying in size according to the race to which they belong. The nuclei, one to four in number, can be easily made out, and have a structure similar to those of the adult and precystic stages. In binucleate and quadrinucleate cysts the nuclear rings often show a crescentic thickening on one side. When chromatoids are present they stain deeply and their presence and shape may be useful in identifying the cyst. The cyst walls are usually invisible in preparations mounted in balsam. (See Fig. 8, Pl. II.)

In stained specimens *E. coli* differs from *E. histolytica* in the following characters. (See Fig. 3, Pl. I.) The cytoplasm is more granular, stains somewhat darker, and usually cannot be separated into ectoplasm and endoplasm. Inclusions are numerous—bacteria, yeasts, &c., which often stain deeply even with haemalum—but no red blood cells are present. Vacuoles, when present, generally have pointed ends and appear as open clefts. The nucleus, which is spherical and usually lies eccentrically, appears, as in *E. histolytica*, as a beaded ring, but the chromatin beads are slightly larger. The karyosome is also larger, 1 μ or more in diameter, surrounded by a very marked halo, and is usually placed eccentrically. Scattered between the karyosome and the peripheral chromatin ring there are always some granules of chromatin.

The precystic stage of *E. coli*, like the same stage of *E. histolytica*, has no inclusions and is in general appearance very similar. The nucleus, however, usually shows a larger eccentrically placed karyosome and the peripheral chromatin ring is composed of larger beads.

Stained specimens of *E. nana* are distinguishable from small forms of *E. histolytica* by the presence of bacteria and other inclusions in the cytoplasm, but chiefly by the nucleus, which is frequently

central in position and only 2 to 3 μ in diameter. Although spherical in shape it appears *not* as a beaded ring, but as a vesicle with a large irregularly shaped karyosome, usually more or less lobed or segmented, lying towards one side. (See Fig. 4, Pl. I.)

Cysts of *E. coli* and *E. nana* are distinguishable from those of *E. histolytica* by the structure of the nuclei and, in the case of *E. coli*, by their number. Further, chromatoid bodies are absent from cysts of *E. nana* and generally appear as granules, filaments, or bundles of spicules when present in those of *E. coli*.

Iodamœba bütschlii (Fig. 6, Pl. I) differs from the preceding species in the structure of its nucleus. This is a small vesicle with a spherical central karyosome which contains all the chromatin present in the nucleus. Between it and the nuclear membrane there is a single layer of granules, which do not stain with chromatin stains, and which are in consequence invisible or inconspicuous unless the preparations have been counter-stained with eosin or some other plasma stain.

The cysts have only one nucleus, which is signet-ring shaped, having a large block of chromatin on one side. The cytoplasm typically shows a clear space with a hard edge where the glycogen mass was situated. (See page 76.)

Dientamoeba fragilis, a very small and rare species, is sometimes found in human stools. It is typically binucleate—its nuclei having a structure which readily distinguishes the organism from those previously described. (See Fig. 5, Pl. I.)

(h) *The Diagnosis of Amoebic Abscess of the Liver or Brain.*

The diagnosis of these conditions is made by finding the active adult form of *E. histolytica*; this should be sought for, *not* in the pus which escapes when the abscess is opened, but in scrapings taken from the walls of the abscess cavity. The material, which is best obtained by means of a blunt spoon, should be examined as soon as possible after its removal from the body, by spreading a small amount on a slide—salt solution being added if necessary—and pressing it out into a thin film by means of a cover-slip. These specimens should be examined systematically with a combination giving a magnification of 300 to 400 diameters.

The amoebae have the same appearance as those found in the stools from cases of acute amoebic dysentery and are recognized by the same characters.

Specimens may be fixed and stained by the method described above (see page 77).

Cysts and precystic forms of the amoebae are not found in the material from such abscesses.

Examination of the faeces may be of considerable help in making the diagnosis of amoebic abscesses, for even though no history or signs of dysentery are obtainable, the cystic or precystic stages, according to the patient's condition, will be found in the stools if careful search is made.

(i) *The Value of Positive and Negative Examinations for the Presence of* E. histolytica *in the Faeces.*

(i) *Acute dysentery.* (*a*) Positive examinations. The discovery of the active adult stage of *E. histolytica* in the stools of a patient suffering from acute dysentery is conclusive evidence that the case is one of amoebic dysentery. It should be remembered that the most important diagnostic character of *E. histolytica*, at this stage, is the presence of red blood corpuscles in the cytoplasm.

The only point to be borne in mind is that 'mixed infections' have been reported in which both *E. histolytica* and *B. dysenteriae* appeared to be concerned in producing the symptoms.

If only the cysts of *E. histolytica* are found in the stools from a case of acute dysentery it is certain that the symptoms are not due to amoebae, for, as has been pointed out above, when amoebae are the cause of acute dysentery the active adult stages are always present in the stools. In cases of amoebic diarrhoea the precystic forms of *E. histolytica* are usually present in the stools in large numbers.

(*b*) Negative examinations. If the examination of suitably prepared specimens made from a freshly passed motion from a case of acute dysentery fails to reveal the presence of the active adult stage of *E. histolytica* it is probable that the case is not one of amoebic dysentery, since, as stated above, this form of *E. histolytica* is usually present in considerable numbers, distributed regularly throughout the blood-stained mucus. A single negative examination is not always, however, a conclusive proof that the case is not one of amoebic dysentery; and it is always advisable to make at least one more examination if the first one has proved negative.

(ii) *The diagnosis of carriers, &c.* In these cases it is usually cysts of *E. histolytica* that are sought for, and if these are found it is certain that the individual harbours *E. histolytica*, and it is also almost certain that he will continue to do so indefinitely, unless some efficient treatment is undertaken. As the cysts are often passed intermittently, a single negative examination is of little value in demonstrating that the individual is not a carrier, and before giving a definite opinion at least six negative examinations should be made.

These examinations may be made at any time convenient to the examiner, as there is no evidence of any periodicity in the passage of the cysts.

(iii) *Examinations made to ascertain if a course of treatment has been successful.* Here again it is chiefly the cysts of *E. histolytica* that are sought for. The examinations, however, instead of being made at any random time, must be carried out according to a definite system. The best routine procedure is as follows: At least six negative examinations are requisite. The first of these is made three or four days after the treatment has been concluded, the second is made one week later—that is, the 10th or 11th day after the cessation of treatment, and the remaining four during the week following that in which the second was made: for instance,

the 18th, 20th, 22nd, and 24th day. The last examination should not be made earlier than the 21st day after the end of treatment.

This system has been devised so that a large proportion of the relapsing cases are picked out by means of the earlier examinations, while as regards the later ones, sufficient time has been allowed to elapse for the infection to return to its usual intensity in those cases where the treatment has temporarily reduced the infection.

Examinations made during the course of treatment are practically useless.

(2) Balantidial Dysentery.

The ciliate protozoon *Balantidium coli*, which occurs commonly in pigs and monkeys, sometimes parasitizes human beings. In such cases it may cause symptoms of diarrhoea and dysentery which are very similar to those produced by *Entamoeba histolytica*. The lesions in the large bowel are also closely alike in balantidial and amoebic dysentery, and differential diagnosis is only possible by microscopic examination of the stools.

Balantidium coli (Fig. 16, Pl. III) is a relatively large, oval organism usually measuring from 50 μ to 70 μ in length, though larger and smaller specimens are sometimes found. It is invested with a delicate pellicle, which appears striated, owing to the presence of longitudinal rows of cilia arising from its surface. In the living state the cilia are in constant movement, and the animal is thus actively motile. The organism is relatively rigid, but capable of passive distortion by the objects among which it moves. It possesses a small funnel-shaped mouth (mo) situated subterminally on the ventral surface at the more pointed anterior end. Another small aperture—the so-called anus—is sometimes visible at the opposite extremity. The protoplasm of the body contains two contractile vacuoles (c.v. 1, c.v. 2) situated in the positions shown in the figure, in addition to a variable number of food vacuoles (f.v.) containing food particles ingested through the mouth. The animal possesses two nuclei—a large kidney-shaped meganucleus (N), and a small spherical micronucleus (n) lying in its concavity. Both usually lie near the middle of the body, and can be made out, with careful focussing, in the living organism.

The cysts are smooth, thick-walled, spherical or slightly oval structures, measuring from 35 μ to about 60 μ in diameter (Fig. 17, Pl. III). In a newly formed cyst the nuclei, the posterior contractile vacuole, and the remains of the ciliary coat can usually be seen. Later, the single organism may undergo further changes, and finally divide within its cyst into two daughter individuals.

Both cysts and free forms may be found in freshly passed stools. Ciliates closely resembling these organisms sometimes occur in the salt solution or water used in diluting the faeces for examination; and this should be remembered as a possible source of error in diagnosis.

The diagnosis of dysentery due to Bilharzia does not come within the scope of this report.

APPENDICES

(1) The Carbinol Test.

(Voges and Proskauer Reaction.)

This test is carried out as follows: The organism is grown for two or three days in broth containing 1 per cent. of glucose. A few cubic centimetres of strong caustic potash solution (20 per cent.) are added and the mixture vigorously shaken to promote oxidation. A positive reaction consists in the development of an eosin-like red colour on standing, often with some fluorescence; this may take some hours to develop, indeed it is best to allow the mixture to stand overnight. The chief organisms yielding a positive carbinol reaction are *B. lactis aërogenes* and *B. cloacae*.

(2) Tests for Indol.

In carrying out any test for indol certain precautions are necessary:

1. The medium in which the organism is grown must contain free tryptophane or a tryptophane polypeptide.
2. The medium should not contain glucose.
3. The medium should always be tested to make certain that it does not already contain indol.

(*a*) *Nitroso-indol Test.* Formerly the test universally employed for the detection of indol was that termed the nitroso-indol test. This consists in the addition of nitrous acid to the culture either by adding a few drops of impure nitric acid (that is, nitric acid containing the lower oxides of nitrogen) or, after the addition of a crystal of nitrite of sodium or potassium, a few drops of strong sulphuric acid. The production of a rose-red colour indicates the presence of indol.

(*b*) *Ehrlich's Test.* Böhme, in 1906, recommmended for bacteriological work a test for indol which had been introduced by Ehrlich. This is carried out by adding to the culture an acid alcoholic solution of paradimethylamidobenzaldehyde and then a few drops of a saturated solution of potassium persulphate. The production of a red colour which can be extracted with amyl alcohol indicates the presence of indol.

A great improvement in this test, due to Steensma, consists in first extracting the indol by shaking with ether.

The test is carried out in the following manner:

The solution of paradimethylamidobenzaldehyde is made by dissolving one gramme in ninety-five cubic centimetres of absolute alcohol and then adding twenty cubic centimetres of concentrated hydrochloric acid.

The bacillus under investigation is planted into an ordinary sized tube containing about seven cubic centimetres of a suitable broth (that described by Cole and Onslow, made by digesting casein with trypsin for a prolonged period, gives excellent results) and incubated at 37° C. for from forty-eight to seventy-two hours. One or two cubic centimetres of ether are added to the culture, which is then shaken up for a few seconds. After the ether has risen to the surface one of two procedures may be followed: the ethereal layer may be pipetted off and placed in a dwarf test-tube containing a few drops of water and a drop or two of the paradimethylamidobenzaldehyde solution carefully added. If indol has been formed, either at once or after standing for a variable time, a rose-red ring of colour develops between the ethereal and watery layers. Alternatively a few drops of the paradimethylamidobenzaldehyde solution may be run down the side of the test-tube after the ether has risen to the surface, when if indol has been formed the rose-red colour develops between the ether and the culture.

The great advantage of ethereal extraction is that no subsidiary colours due to substances other than indol are developed. The addition of an oxidizing solution such as potassium persulphate is not required.

(*c*) *Vanillin Test.* The vanillin test is carried out as follows: A 5 per cent. solution of vanillin is made in absolute alcohol. A few drops of this

having been mixed with about seven to ten cubic centimetres of the culture to be tested, two or three cubic centimetres of a strong hydrochloric acid are added. A deep orange colour is at once produced if indol is present. This colour can be extracted by shaking with amyl alcohol.

Zoller, in a recent paper on the quantitative estimation of indol in biological fluids, has incidentally criticized the comparative value of the three tests for indol mentioned above. His objection to the use of the paradimethylamidobenzaldehyde solution is that it readily reacts with phenol compounds and is slow in developing the maximum colour. The reagent itself also deteriorates on keeping. He also points out that the vanillin test reacts with a large number of compounds of which phenols and ammonia are the chief; further, it is not so discriminating as the nitroso-indol or Ehrlich's reaction.

In the course of his quantitative work Zoller came to the conclusion that the nitroso-indol test is as satisfactory as any for the detection of indol. His method of carrying out this test is to add two drops of a 1 per cent. solution of potassium nitrite to ten cubic centimetres of the liquid to be tested and then five drops of concentrated sulphuric acid. The mixture is shaken and allowed to stand for five minutes. The rose-red colour which develops when indol is present is then extracted with a small quantity of iso-amyl or iso-butyl alcohol.

From the above it would appear that all the tests are open to fallacies but all are useful. The one most usually employed at present is the modification of Ehrlich's test described above and this in the opinion of the Committee is preferable for routine use. In cases of doubt, the organism under investigation should be planted out in 200 to 300 cubic centimetres of broth, and after three or four days' incubation at 37° C. a portion of the culture should be distilled. The distillate is then tested for the presence of indol which has passed over with the water vapour: by this procedure many of the disturbing factors will have been eliminated.

References.

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Steensma, F. A., *Centralbl. f. Bakteriol.* [&c.], I. Abt. Orig., 1906, **41**, 295.
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(3) Brilliant Green Fluid Media.

Peptone water may be used as the basis; this is made by dissolving 2 per cent. peptone with 0·5 per cent. sodium chloride in distilled water, and, after steaming for three-quarters of an hour, filtering through ordinary filter paper. The 'tryptic' broth prepared from casein (Cole and Onslow)[1] is also perfectly satisfactory for this method, as these authors originally suggested. It is important in either case that the medium should have a suitable reaction, which is obtained when, on testing with brom-thymol blue, a greenish tint is obtained (it will be found that at this point brom-cresol purple gives the maximal purple colour, while with phenol-red a tint between fawn and orange is produced). This corresponds to a value of about P_H 7·2 and at this point if a loopful of the medium is placed on red litmus paper a faint blue colour develops in the course of ten to twenty-five seconds. The medium, after adjustment of the reaction, is distributed in amounts of ten cubic centimetres in test-tubes and sterilized by steaming or in the autoclave. A stock solution of 1 per cent. brilliant green in distilled water keeps for many months; from this, immediately before use, a 1 in 10,000 dilution is prepared, and then the necessary amounts are added to the medium, namely 1 in 200,000 in the final dilution for the single tube method; 1 in 400,000, 1 in 250,000 and 150,000 for the three tube method, see p. 39. It has been pointed out (McLeod[2]) that dyes may be supplied under the trade name of brilliant green which do not exhibit the characteristic action on *B. typhosus* and *B. coli*. Therefore 'brilliant green sulphate, crystals, zinc-free'* should be specified, and specimens should be tested for differential antiseptic properties before use (see Browning[3]).

* Works on the chemistry of dyes define brilliant green as a derivative of tetraethyl-diamino-triphenyl-methane.

Telluric acid is stable and keeps indefinitely in the form of a 1 per cent. solution in water, which reacts neutral to litmus. The solution should be sterilized by heat. If employed in combination with brilliant green the strength in the final dilution should be 1 in 25,000, see p. 39.

When a large number of examinations are carried out at one time the mixtures of medium and antiseptics may be prepared in bulk daily and then distributed into sterile plugged test-tubes, which need not contain exactly ten cubic centimetres.

REFERENCES.

[1] COLE and ONSLOW, *Lancet*, 1916, ii, 9.
[2] MCLEOD, *J. Hyg.*, 1919, 18, 260.
[3] BROWNING, *Applied Bacteriology*, London, 1918, 95.

(4) The Preparation of Solid Media for the Isolation of the Enteric Group and Dysentery Bacilli.

(a) *MacConkey's Medium.**

Ingredients required:

Peptone	20 grammes.
Sodium taurocholate (commercial bile salt)	5 „
Agar	20–30 „
Neutral red (1 per cent. solution)	5 cubic centimetres.
Lactose	10 grammes.
Water	1000 cubic centimetres.

The peptone, bile salts, agar, and water are mixed together in a two-litre flask which is then placed in the autoclave and kept at a temperature of 115° C. for forty-five minutes, so as thoroughly to dissolve the various reagents.

In MacConkey's original instructions no mention is made of the necessity for neutralizing the mixture before placing it in the autoclave, but as it may be distinctly acid and it is important not to heat agar in an acid solution, as this tends to destroy its powers to form a firm jelly, sufficient sodium hydrate solution should be added to make the contents of the flask alkaline to litmus.

When the agar and other constituents have been dissolved, the flask is removed from the autoclave, and, after being cooled to 60° C., egg albumen is added to clear the mixture. This should be prepared either by beating up the white of one egg in twenty to thirty cubic centimetres of water, or by dissolving three grammes of dried egg albumen in a similar quantity of water.

The albumen being added, the flask is returned to the autoclave and kept at 115° C. for about ten to twenty minutes. It is then found that the albumen has been completely coagulated and has collected into large masses, leaving the surrounding fluid perfectly clear. This is now filtered through a Chardin's filter-paper and should yield a perfectly clear and bright product. The reaction should now be adjusted to neutrality, P_H 7·0.

The lactose (ten grammes) is dissolved in from ten to twenty cubic centimetres of sterile water in a sterile flask, which should be placed in the steam sterilizer for about fifteen minutes. The lactose solution is then added to the clear agar and thoroughly mixed. Lastly, five cubic centimetres of a freshly prepared sterile 1 per cent. solution of neutral red is added by means of a sterile pipette.

The resulting agar, which is a deep red colour, should be distributed either into large sterile test-tubes containing from twenty to twenty-five cubic centimetres, which is sufficient for a single plate, or into small sterile flasks containing from 100 to 200 cubic centimetres, which is sufficient to make from five to ten average-sized plates. Finally, these tubes or flasks are placed in a steam sterilizer for from twenty to thirty minutes, which is sufficient to ensure sterility if care has been taken to employ the aseptic precautions described above.

* The introduction of neutral red into this medium was due to Grünbaum and Hume, *Brit. M. J.*, 1902, i, 1473

(*b*) *v. Drigalski and Conradi's Medium.*[1]

Ingredients necessary:

Meat	1500 grammes.
Distilled water	2000 c.c.
Agar	70 grammes.
Nutrose	20 ,,
Sodium chloride	10 ,,
Litmus solution	260-300 c.c.
Lactose	30 grammes.
Sodium carbonate (anhydr.) solution, 10 per cent.	4 c.c.
Crystal violet (B. Höchst) solution, 0·1 per cent. freshly prepared	20 c.c.

The meat is minced and extracted in the two litres of cold water overnight; the mixture is then heated and filtered, water, if necessary, being added to the filtrate to make it up to two litres. The peptone, sodium chloride, and agar, having been added to the filtrate, are thoroughly dissolved, as in making ordinary nutrient agar, in the autoclave at 115° C., care being taken that the mixture is alkaline to litmus. This agar is then cleared and filtered into a sterile flask of at least three litres capacity, so that on adding the various reagents thorough mixing can be easily carried out. The reaction having been found to be just alkaline to litmus * the twenty grammes of nutrose are added and dissolved by placing the flask in the steam sterilizer for ten minutes after thoroughly shaking. (It is convenient to have a stock of agar of this sort as it keeps well.) The litmus-lactose solution is now prepared. This can be done in several different ways, that most generally recommended being to place 260 c.c. of litmus solution (Kahlbaum's) in a sterile flask; after this solution has been steamed for ten minutes, the lactose (thirty grammes) is added and dissolved in the hot liquid. The flask is again placed in the steam sterilizer for fifteen minutes to ensure sterility.

If Kahlbaum's litmus solution is not available, a carefully prepared, saturated, watery solution of litmus, which has been freed as far as possible from all traces of litmus red, should be procured from some good commercial firm, or such a solution may be prepared in the following manner:

Solid commercial litmus is digested with pure spirit at 30° C. until, on adding fresh alcohol, the latter becomes only a light violet colour. This is best done by placing the litmus in a flask and pouring the alcohol over it; the flask is then closed with a rubber cork and placed in an incubator at about 30° C. The flask is well shaken at intervals. After a day or two's incubation the alcohol is poured off and fresh added; this is continued until no more red violet colour is extracted by the alcohol. The alcohol is now filtered off and the residue, after being dried, is dissolved in distilled water to saturation. This solution on being diluted with distilled water should give a pure blue colour. To this saturated solution of litmus enough sulphuric acid (1 in 1,000) is added to produce the slightest change of colour. The solution is then ready for use.

Having dissolved the lactose in the litmus in the manner described above, the mixture is added to the nutrose agar, which is thoroughly shaken, and, if necessary, the reaction is adjusted until the froth takes on a violet-blue colour. Four c.c. of a hot sterile 10 per cent. solution of anhydrous sodium carbonate and 20 c.c. of a 0·1 per cent. freshly prepared solution of chemically pure crystal violet are then added and the mixture is again thoroughly shaken. The agar is then distributed into small flasks of 100 to 200 c.c. capacity, these containing sufficient medium to make from 5 to 10 medium-sized plates; or in quantities of 20 to 25 c.c. in large test-tubes, this being sufficient for a single plate. This distribution is preferably carried out with aseptic precautions, the tubes or flasks being previously carefully sterilized; a few minutes (10 to 20) in the steam sterilizer is then sufficient to ensure the sterility of the medium.

(*c*) *Endo's Medium.*[2]

Ingredients required:

Nutrient agar, 3-3½ per cent.	1000 c.c.
Sodium carbonate (cryst.) solution, 10 per cent.	6 c.c.
Lactose (pure)	10 grammes.

* See foot-note on p. 88.

Alcoholic solution of basic fuchsin, saturated * . .	5 c.c.
Sodium sulphite (cryst.) solution, 10 per cent. . .	25 c.c.

Preparation:

Into a sterile 2-litre flask the 1,000 c.c. of 3 to 3½ per cent. nutrient agar is placed and the reaction is tested to ascertain that it is just alkaline to litmus.† If the reaction has to be adjusted by the addition of either acid or alkali, the agar should be placed in the steam sterilizer for a quarter of an hour. Should this produce any precipitate, the agar must be filtered and the reaction again tested. When this is satisfactorily accomplished the 6 c.c. of the 10 per cent. solution of crystallized sodium carbonate are added, and the mixture, after being thoroughly shaken, should be again placed in the steam sterilizer for 20 minutes. The 10 grammes of lactose are dissolved in a small quantity of sterile water in a sterile flask. The solution of sodium sulphite is prepared by dissolving 2·5 grammes of crystallized sodium sulphite in 25 c.c. of sterile distilled water with the aid of heat, but without allowing the water to boil, and the saturated alcoholic solution of fuchsin should be filtered.

The agar having been removed from the steam sterilizer, first the lactose solution is added, then by means of a pipette the saturated alcoholic solution of basic fuchsin, thorough mixing being assured by vigorous shaking between the addition of each ingredient. Lastly, the sodium sulphite solution is thoroughly mixed into the agar. The addition of the fuchsin solution produces a deep red colour which is slowly bleached by the sodium sulphite solution, so that by the time the agar has been distributed into large-sized test-tubes (20 to 25 c.c. in each) or small flasks holding 100 to 200 c.c. (sufficient to make 5 to 10 medium plates) and has cooled, the colour has almost completely faded. The medium should be kept in the dark, and should not be melted except on the single occasion necessary for making the plates. In consequence, all tubes and flasks into which it is distributed should be sterilized and aseptic precautions should be taken in carrying out these manipulations, thus avoiding the necessity of fractional sterilization.

(*d*) *Meyer and Stickel's modification of Holt-Harris and Teague's Medium.*[3]

Ingredients required:

Bullock's heart or an equal weight of lean beef.
Some active form of trypsin.
Sodium carbonate.
Acetic acid.
Calcium chloride.
Lactose.
Aqueous solution of yellowish eosin, 2 per cent.
Methylene blue solution, 0·5 per cent.
(Quantities of the ingredients required are given in the text.)

A stock agar is first prepared from broth made by digesting beef or bullock's heart with trypsin as described by Douglas. This is done as follows: A bullock's heart cleaned of all fat, vessels, &c., is minced finely, or, if more convenient, an equivalent amount of lean beef or horse-flesh may be substituted for the heart muscle. To the meat, tap-water in the proportion of one litre of water to 500 grammes of the mince is added, together with sufficient sodium carbonate solution to render the mixture distinctly alkaline. The trypsin is added in sufficient quantity to bring about a rapid digestion of the meat; for instance, when the Inj. trypsinae co. (Allen and Hanbury) is employed 10 to 15 c.c. is sufficient. Digestion is allowed to continue for from 2½ to 3 hours; the vessel containing the mixture being kept warm either by placing in a hot room at 37° C. or in a water-bath having a temperature of 45° to 55° C.

After the digestion has been carried far enough ‡ the mixture is brought to

* Half this amount of fuchsin will generally give equally good or better results.

† See foot-note on p. 88.

‡ To ascertain if the mixture has been sufficiently digested a sample should be tested for the presence of free tryptophane. This is carried out by cautiously adding bromine water to a filtered sample of the digest rendered acid with acetic

the boil and acidified with acetic acid, which causes all the coagulable protein to be precipitated.

The mixture is allowed to stand until the suspended matter has had time to settle and is then strained through several thicknesses of muslin. Calcium chloride in the proportion of from 0·25 to 0·5 grammes per litre is then added and when dissolved the reaction is adjusted to P_H 7·0 or P_H 7·2.* Two per cent. agar is then added and the mixture autoclaved from 30 to 45 minutes at 110° to 115° C. to ensure thorough solution of the agar. On cooling to 60° C. white of egg is added in the proportion of the white of one egg or 3 grammes of dried egg albumen dissolved in a convenient quantity of water to each litre of agar. The flask is again autoclaved at a temperature of 115° C. for about 10 to 20 minutes and then filtered through a Chardin's filter paper. Ten grammes of lactose dissolved in a little sterile hot water are now added to each litre of the clear agar and the reaction is verified and, if necessary, adjusted to P_H 7·0. This forms the stock agar which keeps indefinitely and is best distributed into small flasks containing 50 or 100 cubic centimetres which should be sterilized by steaming.

The 0·5 and 2 per cent. solutions of methylene blue and eosin are prepared separately by dissolving respectively half a gramme and two grammes of the solid stains each in 100 cubic centimetres of hot sterile distilled water contained in sterile flasks. When completely dissolved the solutions should be steamed for thirty minutes to ensure sterility and then stored in sterile bottles fitted with rubber corks or caps.

To prepare the plates, the lactose agar having been melted, to each fifty cubic centimetres one cubic centimetre of the solution of eosin is added by means of a sterile pipette and then one cubic centimetre of the methylene blue solution. After thorough mixing the plates are poured in the usual manner.

References.

[1] v. Drigalski and Conradi, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1902, 39, 283.
[2] Endo, S., *Centralbl. f. Bakteriol.* [&c.], I. Abt. Orig., 1904, 35, 109.
[3] Meyer, K. F., and Stickel, J. E., *J. Infect. Dis.*, 1918, 23, 48.

(5) Fluid Media used for the Isolation of *V. cholerae*.

(*a*) *Peptone water.* (*Dunbar*)

Reagents required:

Peptone	10 grammes.
Sodium chloride	5 ,,
Sodium nitrate	0·1 ,,
Water	1,000 cubic centimetres.

These reagents are placed together in a flask and steamed until thoroughly dissolved. The solution is then filtered and the reaction adjusted to between P_H 8·0 and P_H 9·0. The solution is again steamed for about thirty minutes and, if any precipitate is formed, it should be filtered off and the reaction of the fluid verified. Finally it is distributed into tubes or small flasks and sterilized at 110° C. for twenty minutes in the autoclave.

When drinking-water is examined for the presence of *V. cholerae* the peptone water is usually made up in a ten times more concentrated solution.

acid, when, if tryptophane is present, a rose-red colour will be produced which may be extracted with amyl alcohol. The colouring matter turns to yellow on adding an excess of bromine. The presence of but a slight positive reaction indicates that the digestive process has been carried far enough.

* The three media first described were introduced before exact methods had been described by which the reaction of such mixtures could be easily estimated. Now, however, simple methods are available, by which the hydrogen-ion concentration can be determined, such as those described in the Medical Research Committee's Special Report Series, No. 35, entitled 'Reaction of Media'. It is therefore advisable that the agar used in their preparation should be adjusted to a definite reaction such as P_H 7·0 before the addition of the dye solution. The addition of a known quantity of sodium carbonate to Drigalski and Conradi's and to Endo's media would under these conditions be unnecessary.

Nine volumes of the water under examination are then placed in a sterile flask and one volume of the concentrated sterile peptone solution is added. After the flask has been incubated at 37° C. the resulting growth is examined in the usual manner.

(*b*) *Goldberger's Alkaline Egg Broth.*[1]

This medium is stated by Goldberger to be as selective as peptone water, but it gives even better results than the latter when the vibrios are few in number. It is prepared in the following manner: One egg is broken into a sterile beaker and the yolk and white intimately mixed with an equal volume of water. A volume of 5 per cent. anhydrous sodium carbonate solution equal to the egg and water mixture is added and thoroughly mixed. The beaker is then placed in the steam sterilizer for from forty-five minutes to one hour.

One volume of this alkaline egg mixture is added to nine volumes of ordinary neutral peptone water. The alkaline egg broth is then distributed into tubes or flasks and sterilized by steaming. The concentrated alkaline egg mixture keeps for several weeks, and when diluted with peptone water will keep for at least seven days.*

REFERENCES.

[1] GOLDBERGER, J., *Bull. Hyg. Lab.*, Washington, 1913, No. 91, 19.

(6) Solid Media used for the Isolation of *V. cholerae.*

(*a*) *Dieudonné's Medium.*[1]

Ox blood is obtained from the slaughter-house and at once defibrinated. This is best done by receiving the blood direct from the animal into a sterile vessel and then stirring with glass rods until all the fibrin has been removed. Immediately the blood reaches the laboratory it should be mixed with an equal volume of normal potassium or sodium hydrate solution, which produces a reddish black almost translucent solution. After distribution into sterile flasks of suitable size the solution should be steamed for about thirty minutes.

To prepare the finished medium three volumes of this blood alkali mixture are mixed with seven volumes of any ordinary neutral nutrient agar * which has been melted in the steamer, and the plates are at once poured. Before being planted the plates should be kept at room temperature for at least twenty-four hours after any water of condensation has been dried off in the incubator.

Alternative methods for rendering the plates ready for sowing are (1) to place them in a bell-jar containing carbon dioxide gas for thirty to sixty minutes; (2) to store the blood-alkali mixture in a flask plugged with cotton wool for six or eight weeks before use; (3) to substitute an 11·4 per cent. solution of andydrous sodium carbonate for the normal solution of caustic alkali. All these methods give excellent results and the second method shows that the blood-alkali mixture keeps perfectly and even improves for at least two months. If, however, it is kept for a longer period than two months, it should, after this period, be protected from free contact with the air by substituting a rubber bung for the cotton wool plug, otherwise the selective properties of the medium become impaired.

(*b*) *Goldberger's Alkaline Egg Agar modified by Douglas.*

An egg is broken into a sterile beaker and the contents thoroughly mixed with an equal volume of water. A volume of 6·5 per cent. solution of anhydrous sodium carbonate solution equal to that of the egg and water mixture is then added. The mixture is then steamed for from thirty minutes to one hour. This alkaline egg mixture keeps for three months at least. Any ordinary neutral nutrient agar * is melted and 1 per cent. of cane sugar is added to it.

* In a series of experiments carried out by one of us the nutrient agar used in making up both Dieudonné's and Goldberger's media was a trypsin-digest agar prepared in the manner recommended by Douglas. The results obtained were excellent. S. R. Douglas, *Lancet*, 1914, ii. p. 891.

One volume of the alkaline egg mixture is then well mixed with five volumes of the neutral cane-sugar agar and, immediately before the plates are poured, 1 per cent. of a freshly prepared 0·5 per cent. solution of neutral red is added. This medium, which has an orange tinge, when planted with *V. cholerae* gives colonies which after twenty-four hours' incubation at 37° C. have deep red centres.

REFERENCE.

[1] DIEUDONNÉ, *Centralbl. f. Bakteriol.* [&c.], I. Abt. Orig., 1909, 56, 107.

(7) The Haemolytic Test for the Differentiation of *V. cholerae* from Allied Vibrios.

This test should always be carried out by mixing definite volumes of a broth-culture of known age with an emulsion of washed red cells; the red cells usually employed being either sheep's or goat's. Tests made by planting the vibrio under examination on to blood-agar plates are not reliable.

A broth culture of the vibrio should be made and incubated for three days at 37° C. The culture should then be cleared of organisms by centrifuging in a high speed centrifuge for a long period, or by being placed in the ice chest for some days. Filtration absorbs so much of the haemolytic substance that it should never be employed. The clear supernatant fluid is pipetted off and only this is used for the test.

An emulsion of sheep's red blood corpuscles (if more convenient rabbit's or human red cells may be used) is prepared by repeatedly washing in physiological saline sufficient red cells to make up a suitable quantity of a 5 per cent. emulsion. This is best done by centrifuging a quantity of defibrinated sheep's blood, pipetting off the serum, resuspending in physiological salt solution and again centrifuging. This process is repeated according to the size of the centrifuge tubes until every trace of serum has been removed.

Into a series of small test-tubes (3 in. × $\frac{3}{8}$ in.) one cubic centimetre of a dilution or of undiluted culture is first added, the usual dilutions employed being, into the first tube one cubic centimetre of undiluted culture;[1] into the second, one cubic centimetre of a 1 in 2 dilution; into the third, one cubic centimetre of a 1 in 10 dilution; into the fourth, one cubic centimetre of a 1 in 20 dilution; and into the fifth, one cubic centimetre of a 1 in 100 dilution. Control tubes containing respectively one cubic centimetre of physiological saline and one cubic centimetre of the broth in which the culture was grown should always be added. To each of these tubes one cubic centimetre of the 5 per cent. emulsion of red blood cells is then added and the contents thoroughly mixed; this is most easily carried out by bubbling air through the contents by means of a capillary pipette. The tubes are incubated at 37° C. for four hours and then placed in the ice chest overnight and examined for haemolysis on the following morning.

REFERENCE.

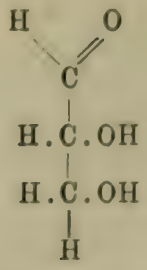
[1] GREIG, E. D W., *Indian J. M. Research*, 1914, 2, 623.

(8) Sugars in their Relation to Fermentation Reactions.

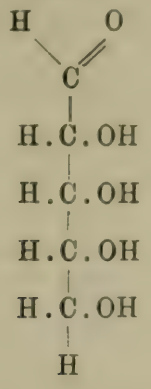
Although sugars have been used in bringing out differences in the cultural behaviour of micro-organisms for many years, the mechanism of these reactions is still very imperfectly understood. There are certain suggestive features in the constitution of these compounds which help to explain the selective action of bacteria upon them, and also show why it is necessary or unnecessary to use certain sugars when testing fermentative activity.

The sugars are divided into three great classes: (1) the monosaccharides; (2) the disaccharides; (3) the polysaccharides including the tri- and tetra-saccharides. This classification is based on a unit formula containing seven carbon atoms or less.

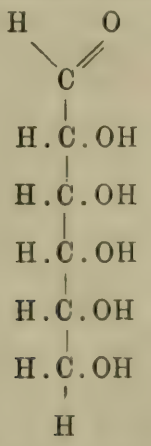
For example, a triose, glycerose, has a chain of three carbon atoms,



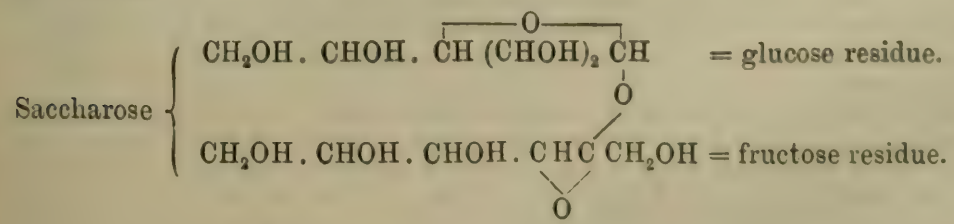
a pentose, five carbon atoms, e.g. arabinose:



a hexose, glucose (dextrose), six carbon atoms:



The disaccharides are sugars in which two monosaccharides have been linked together to form a single compound. This combination takes place with the elimination of a molecule of water. Hence, when these compounds are 'hydrolyzed' or split up in aqueous solution, as may happen by heating with weak acids, or by the action of digestive or bacteriological ferments, two molecules of monosaccharides are formed. Thus a molecule of saccharose yields one molecule of glucose (dextrose) and one molecule of fructose (laevulose).



$$+ H_2O =$$

$CH_2OH.CHOH.CHOH.(CHOH)_2.CHO$ = glucose.

$+ CH_2OH.CHOH.CHOH.CHOH.CO.CH_2OH$ = fructose.

Lactose yields one molecule of glucose and one molecule of galactose. Similarly the polysaccharides, split up into various sugars depending on the compound employed and the method of hydrolysis. The colloidal polysaccharides, of which starch is an example, are composed of a number of monosaccharide groups. The number is uncertain, but a recent determination goes to show that at least seven hexose groups are present in the starch molecule.

The following is a classification of the sugars:

1. Monosaccharides.	Dioses.	Aldose	Glycolaldehyde.
	Trioses.	Aldoses	Glycerose.
		Ketoses	Dioxyacetone.
	Tetroses.	Aldoses	Erythrose.
		Ketoses	d. Erythrulose.
	Pentoses.	Aldoses	Arabinose, xylose, ribose.
		Ketoses	l. Arabinulose.
	Hexoses.	Aldoses	Glucose, galactose, mannose.
		Ketoses	Fructose, sorbose.
	Heptoses.	Aldoses	d. Mannoheptose.
2. Disaccharides.		Lactose	Glucose + galactose.
		Maltose	Glucose + glucose.
		Saccharose	Glucose + fructose.
		Trehalose	Glucose + glucose.
		Melibiose	Galactose + glucose.
3. Trisaccharides.		Melitose (Raffinose).	
		Melizitose	Fructose + glucose + galactose.
4. Tetrasaccharides.		Lupeose.	
		Stachyose.	
5. Polysaccharides (colloidal)		Dextrins.	
		Glycogen.	
		Cellulose.	
		Starch.	
		Mucilages.	
		Gums.	
		Inulin.	

It will be noted that in the case of a disaccharide such as trehalose that it contains two molecules of glucose and is in this respect similar to maltose. The difference in structure between these two sugars is due to the way in which the two glucose molecules are linked together by the atom of oxygen. This also applies to the pair lactose and melibiose, which contain a molecule of galactose and glucose.

It will also be remarked that inosite, the so-called 'muscle sugar', which has been used in bacteriological tests, is absent from the list. This compound, although having the formula $C_6H_{12}O_6$, is not a sugar, but a hexahydroxymethylene compound which has only slight affinity with a carbohydrate formula, and hence should not be included in a list of carbohydrates, although it is frequently fermented by bacteria with the production of acid and gas.*

The bioses, trioses, and tetroses have rarely been employed in testing fermentation activity, but the higher members of the series, from the pentoses to the colloidal polysaccharides, have all been employed at one time or another for this purpose.

The fundamental point in the sugars which confers on them their usefulness is their molecular (structural) complexity; for in this complexity the enzymes of a given micro-organism are able to interlock as do the wards of a complicated key, and perform the initial dislocation of the molecule which eventually leads to its complete destruction. The complexity of the sugar molecule is in part due to the fact that it contains a number of asymmetric carbon atoms, and the

* Rhamnose (isodulcite), which is referred to on page 44, is a methyl-pentose and cannot be strictly included in the above grouping.

spatial arrangement of the groups around each atom determines what the sugar will be without any further change in the structure of the molecule being necessary.

In order to realize this conception it is necessary to remember that the groups attached to an asymmetric carbon atom must all be dissimilar, and that the arrangement of these groups in space may be such that one compound is exactly the mirror image of the other, just as a left-hand glove containing the same number of fingers and a thumb as the right is the mirror image of the latter. The two gloves differ from one another in only one particular, viz. that it would be impossible to place one glove above another with both palms upward and have the thumbs in contact.

Hence the optically isomeric sugars would contain the same groups in the same positions, differing only from one another as a right-hand glove does from a left. The permutations, which may occur, are able to produce in a carbohydrate formula containing four asymmetric carbon atoms, of which glucose is an example, sixteen sugars. So that for a given general formula containing four asymmetric carbon atoms a micro-organism may have a choice of sixteen with which to interlock its enzymes of peculiar structure. This is often found to be the case, for in the language of the bacteriological laboratory it is said that a given bacterium will ferment glucose, but not galactose, although both these sugars are aldehydes and would yield an identical formula on ultimate analysis.

The matter of the spatial relations of the groups around the asymmetric carbon atoms is further complicated by the sugars having two end groups of different structure. In one group of sugars, the aldoses, the termination of one end of the chain is a $-C\begin{matrix}H\\ \diagup\\ \diagdown\\ O\end{matrix}$ group, characteristic of all aldehydes, while in the ketoses, of which fructose (laevulose) is an example, the second carbon atom in the chain is united by a double bond to oxygen to form the group $\begin{matrix}|\\ C=O\\ |\end{matrix}$ which is found in all ketones.

Another feature of these compounds, and one that is somewhat disturbing from the bacteriologist's point of view, is their lability. By the solution of these substances in water, one compound tends to change to another owing to the unstable character of the terminal groups.

Thus ordinary glucose in alkaline solution gradually and partly changes to d. fructose and d. mannose. Eventually a state of equilibrium is reached in which a definite proportion of each is present. Hence a fermentation test obtained with glucose cannot be ascribed to that substance alone, but may have arisen from one or both of the other sugars. Conversely, if a positive test is obtained with glucose, it is of necessity useless to employ fructose or mannose for further tests, for both these sugars will undoubtedly be fermented.

The transformation of the corresponding alcohols by this tautomeric change does not take place, for these compounds do not have the $-C\begin{matrix}O\\ \diagdown\\ \diagup\\ H\end{matrix}$ and $\begin{matrix}|\\ C=O\\ |\end{matrix}$ groups possessed by the sugars. They should, therefore, be of great value in differentiation. Unfortunately, with the exception of dulcitol, mannitol, and sorbitol (mannitol, the alcohol corresponding to d. mannose; sorbitol, the alcohol corresponding to d. glucose or d. fructose; dulcitol, the alcohol corresponding to galactose), they are exceedingly difficult to obtain, and have, therefore, not had the prominence in fermentation reactions which they deserve.

When glucose is treated with methyl alcohol in the presence of hydrochloric acid a methyl ester (salt) of the sugar is formed, showing that glucose and similar compounds have the property of combining with other substances to form ester-like compounds.

This is a very common property of sugars, and one may look on maltose, for example, which contains two molecules of glucose as the glucose ester of glucose, but the property of combining goes much farther afield and gives us a class of compounds which are of great importance in bacteriology and in medicine.

These are the glucosides. Amongst the sugars themselves maltose is looked upon as a glucose-glucoside, lactose as a glucose-galactoside. Plants

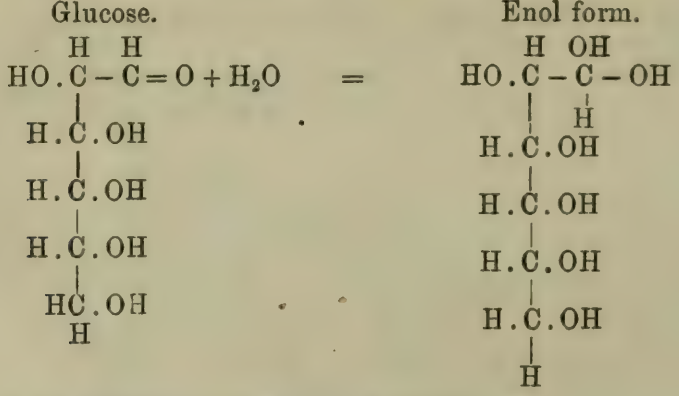
have a special faculty of combining sugars with other substances, such as hydroquinon, indoxyl, benzoic acid, and many others of much more complicated structure. We already know more than a hundred natural glucosides, only a few of which, e.g. salicin, arbutin, aesculin, have been used in bacteriology. If these substances were available in quantity, it is likely that some of them would render service in differentiating bacteria.

The mechanism whereby bacteria attack carbohydrates is a matter into which hypothesis largely enters. It is by no means so well substantiated by experiment as the degradation of proteins. The compounds which are the supposed first products of attack are so labile as to escape present methods of detection.

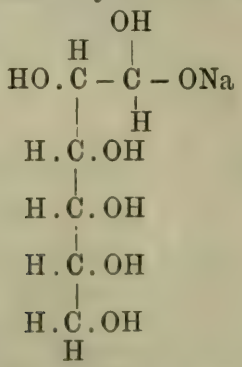
In the first place one must suppose that between an aldose (e.g. glucose) and its corresponding ketose (fructose) there is a compound formed by the addition of water to either. This is the 'enol' compound, containing two hydroxyl (– OH) groups attached to one carbon atom. Such compounds are always most unstable and are capable of being attacked readily. The formation of an enol compound takes place most easily in alkaline solution. This fact has important bearing on the reaction of a medium and its behaviour to a sugar when the latter is heated in it, and will be referred to later.

As has been said, the enol compound is liable to attack by any agent, however feeble, and splits at its places of least resistance—the double bond—into pieces containing a varying number of carbon atoms. From these pieces the acids (formic, acetic, lactic) and other similar compounds are formed.

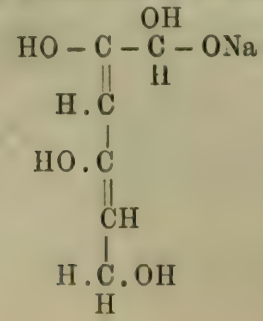
A schematic diagram of the process above described, using glucose as an example, would be the following:



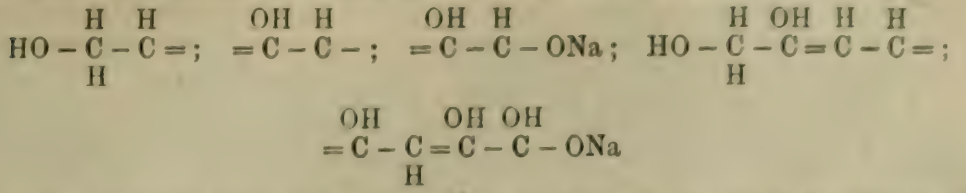
This in an alkaline solution may form the salt



This loses water with the formation of double bonds after forming



and, under the influence of bacterial enzymes, breaks up into pieces such as



It will be seen from the above scheme that the possibilities of fragmentation are great, and we can infer that the acids, alcohols, and other substances formed, which are found as the result of the action of these enzymes, are due to the utilization of these fragments for subsequent synthesis. The complexity of the fragments and the numerous points of attack also indicate to what an extent an enzyme may have the opportunity of exerting its peculiar and specific property of producing a special compound which is not found as a result of the action of other ferments of a related type.

One of the substances resulting from the fragmentation which is of importance in the differentiation of bacteria of the colon group is acetyl-methyl carbinol $CH_3.CHOH-CO.CH_3$ which has been shown by Harden and by Lemoine to give rise to the Voges and Proskauer reaction.

In the fermentation of sugar by the organisms of the *B. coli* group two of the important products found are acid and gas. The acids produced are of various kinds, of which lactic, acetic, and formic acids may be mentioned. The gases formed consist of hydrogen and carbon dioxide.

According to Harden the equation may be represented as—

$$2C_6H_{12}O_6 + H_2O = CH_3COOH + 2CH_3.CHOH.COOH + C_2H_5OH + 2CO_2 + 2H_2$$

It is believed that three bacterial enzymes take part in the reaction. The first enzyme forms lactic acid—

$$C_6H_{12}O_6 = 2CH_3.CHOH.COOH$$

The second produces alcohol and acetic and formic acids—

$$C_6H_{12}O_6 = C_2H_5OH + CH_3COOH + 2H.COOH$$

The third enzyme decomposes formic acid into carbon dioxide and hydrogen—

$$H.COOH = CO_2 + H_2$$

These three equations only represent principal reactions, but many subsidiary processes take place yielding other compounds.

To recapitulate what has been said in the foregoing paragraphs:

(1) The carbohydrates owe their usefulness in the differential diagnosis of bacteria to slight differences in structure which permit certain bacterial enzymes only to react with them.

(2) A given carbohydrate, when once attacked, may break down in many ways, yielding a variety of substances, alcohols, acids, and compounds of mixed structure. Some of these compounds may be of diagnostic value, as, for example, the acetyl-methyl carbinol, encountered in the Voges and Proskauer reaction.

(3) The stability of carbohydrates in solution is not great, and hence a certain amount of stereoisomers is always found when they are dissolved in water. For this reason, one of the stereoisomers is always sufficient in a fermentation test. When this is positive, it is certain that the companion sugar will give a positive reaction.

The Purity of the Carbohydrates.

The commercial samples of carbohydrates, with the exception of saccharose and possibly lactose, have not been above suspicion. Particularly has this been the case with glucose, which has often been supplied in a form which yields a brown solution with water, and which is obviously impure. No guarantee could be obtained that the rarer sugars were of a quality sufficiently good for the delicate differences which bacteriological work imposes upon them. Undoubtedly some of the differences in fermentation reactions found by investigators have been due to the unequal qualities of the carbohydrates employed. Recently certain American firms have undertaken to supply sugars of tested purity, and those samples which have come to hand have the appearance of very pure products. This work has been encouraged by the Carbohydrates

Laboratory of the U.S. Department of Agriculture, where methods for refining the sugars have been worked out.

For the purification of glucose, fructose, xylose, and other sugars, the method of crystallization from acetic acid solution can be confidently recommended. The sugars so obtained give an absolutely water-white solution when dissolved, and a single recrystallization will yield a product of greater purity than can usually be obtained commercially. The method for glucose is very completely described by Hudson in the *Journal of the American Chemical Society*, 1917, **39**, 291.

The Effect of Sterilization on Sugars dissolved in a Medium.

Many years ago Hesse pointed out that a medium containing a sugar became more acid after sterilization when tested by ordinary bacteriological titration, and this has been repeatedly confirmed by other workers. It is also a matter of common experience that one never obtains a medium in which a carbohydrate has been sterilized which is so light in colour as the same medium made without the sugar.

Both the increase in the acidity and the deepening of colour are due to reactions in which the sugar plays a part. With regard to the increase in acidity, it has been shown that a sugar which is dissolved in a medium which is alkaline tends to change to the sodium compound of the enol configuration. This salt is unstable, and especially when the temperature of the solution is raised, as it is in sterilizing, the sugar molecule breaks at various points and produces a series of acid compounds of which saccharic acid is the highest, and traces of acetic and formic acid represent the simpler. Due to this the medium becomes more acid. Unfortunately it is not possible to prevent this increase in acidity by making the medium more alkaline before sterilization, for owing to the increase in hydroxyl ions the changes are accelerated and one obtains more acid products than ever.

The drop in alkalinity depends on many factors, the concentration of hydroxyl ions, the content of sugar, the temperature to which the medium has been raised, and the time of sterilization being the chief. It is not at all unusual in a glucose peptone medium which has been adjusted to P_H 7·4 before sterilization to have a final reaction of $P_H = 6{\cdot}8 - 6{\cdot}9$. Such changes in reaction may be of some importance where strict comparisons of fermentative activity are being carried out, and should be avoided, if possible. The second reaction which is denoted by the increase in depth of colour, when sugar-containing media, especially those made with peptone, are sterilized, is due to a peculiar and very interesting reaction which has been studied extensively by Maillard. In this reaction, carbohydrates combine with amino-acids to form brown substances of extremely complicated structure, and it is to a reaction of this kind that humus formation in soils is possibly due. Among the amino-acids which are particularly liable to this reaction are tryptophane and histidine. As the former is the amino-acid concerned in the indol test, it is important that any disturbance of its concentration in a medium should be avoided.

The only method that can be safely recommended is to adjust the sugar-free medium to the required reaction and sterilize it. In this way very little change in the reaction will take place. The sugar solutions are made up in distilled water in concentrations of 10 to 20 per cent. and sterilized separately either by heating or by passing through a sterile porcelain filter. The concentrated sugar solution is then added to the medium in the necessary quantity with a sterile pipette.

If the sugar solutions are sterilized by heat it is advisable in the case of glucose to add just sufficient acid to the solution to make it faintly acid to congo red paper. With lactose or saccharose solution the addition of acid is not necessary.

Part II. THE PRINCIPLES AND PRACTICE OF THE AGGLUTINATION TEST, WITH SPECIAL REFERENCE TO THE GROUP OF INTESTINAL BACILLI.

I. Introduction.

Amongst the various serum reactions which are available for determining the specific nature of a bacterium on the one hand, or of an infective disease on the other, the simplest and most convenient is agglutination.

The clumping of bacteria by an immune serum had been incidentally noticed by more than one observer before 1896, but it was in this year that the phenomenon was first seriously studied by Gruber and Durham. It was shown to be a specific reaction, by the aid of which an unknown bacterium could be recognized and differentiated from allied species. Gruber and Durham's work was concerned chiefly with the cholera vibrio, the typhoid and the colon bacilli, and with the diagnosis of the bacterium, but shortly afterwards Widal and Grünbaum, independently, showed that the reaction might equally be employed in the reverse direction for the clinical diagnosis of the disease in the case of typhoid fever.

It is now well established that the property of agglutination by a specific serum may afford valuable help in either direction; where a known specific serum is available it may be used for the diagnosis of an unknown bacterium; where emulsions of known bacteria are in readiness, for the recognition of a specific antibody in the blood of a patient and thus of the disease. No method in clinical pathology has proved of greater practical utility, for it has been found available in a considerable number of bacterial diseases. Its application has not only enabled us to discriminate between allied bacterial species, but has in some cases revealed the presence, within the limits of a single species, of 'serological races' or subspecies, which behave as separate entities and may require separate measures in prophylaxis and treatment. The property of being agglutinated is not confined to bacteria; red blood corpuscles may be clumped by a suitable immune serum; but it is with the diagnosis of bacteria and of infective diseases that we are here chiefly concerned.

(1) The Phenomena of Agglutination.

When a person is attacked by certain infective diseases it is commonly found that in the course of a week or ten days his serum either acquires a property which it did not possess before, or exhibits it in an enhanced degree. When mixed with a uniform suspension of the infecting organism it causes the bacteria to run

together and adhere in larger or smaller clumps. Similarly, when an animal, such as a rabbit, receives an injection of a pure culture of certain bacteria, its serum speedily acquires the same property. So long as the injection does not kill it it is a matter of indifference whether the bacteria introduced are living or dead, or whether the dose be administered by the subcutaneous, intravenous, or intraperitoneal route. By repeating the injection at suitable intervals the agglutinating power of the animal's serum may be raised to so high a point that it is manifested even when the serum is diluted many thousand times.

The agglutination of bacteria may be observed in two ways, under the microscope or with the unassisted eye. If a young broth culture, or a uniform saline suspension of a young agar culture of the typhoid bacillus, is mixed with suitably diluted typhoid immune serum, and the mixture watched under a magnification of a few hundred diameters, either as a hanging drop or on a plain slide under a cover glass, the following sequence of events is observed. At first the individual bacilli are seen in active motion and uniformly distributed throughout the field; but after an interval, which is shorter or longer according to the potency of the serum in the dilution employed, the movements are seen to become less active and finally to cease. Frequently progressive movement is replaced for a while by a spinning action about a stationary axis. As motility ceases, individual bacilli are seen to approach and adhere to one another, at first by twos and threes, but presently, by the accession of fresh individuals or the cohesion of adjacent groups, in clumps which increase till they number hundreds or thousands of bacteria. The clumps, at first loosely aggregated, become finally more compact, while the intervening areas of fluid become more and more free from bacteria till all have been included in the clumps. The agglutination is now said to be complete. In the case of a non-motile organism the process is the same, except as regards initial loss of motility.

A similar mixture of bacterial emulsion and immune serum, in larger volumes, placed in a small test-tube in an incubator will show the same phenomenon on a larger scale so that it is plain to the naked eye. The suspension, at first homogenous, becomes granular in appearance and then finely floccular. The flocculi gradually increase in size till they resemble snowflakes floating in a clear fluid: in time they gravitate to the bottom of the tube, leaving the supernatant fluid practically clear: sedimentation by gravity is the final result of agglutination.

The bacteria are not killed by being agglutinated, unless there is concomitant bacteriolytic action on the part of the serum. It is easy to prove that agglutination and bacteriolysis are separate phenomena by heating an immune serum to 55° C. for half an hour and then testing it on a suspension of the corresponding bacterium. The complement being destroyed by the heating, no bacteriolysis can occur, but agglutination takes place as before, and the sedimented bacteria are found to grow readily on culture.

If an immune serum be progressively diluted a point is reached at which its agglutinating power ceases to be manifested. This

end point measures the potency of a serum and is called its 'end-titre', or, more shortly, its 'titre'. If a serum agglutinates its corresponding bacterium in a dilution of 1 in 4,000, but fails to do so distinctly at 1 in 6,000, we say that its titre is 1 in 4,000, or, more shortly, 4,000. Sera may be produced with a titre as high as 1 in 20,000, or even 1 in 100,000—that is to say such a serum is still active even when diluted 20,000 or 100,000 times. In measuring the end-point some arbitrary degree of agglutination must be taken as a criterion—such for instance as complete sedimentation on standing or a point at which the flocculi are just perceptible to the naked eye.

The power of agglutination conferred upon the serum by an attack of infective disease, or an injection of the specific bacterium, is not permanent. If carefully measured agglutination titres be plotted from day to day on a chart the curve is found rapidly to rise to a maximum which, in the case of an uncomplicated attack of typhoid fever in an uninoculated man, is usually reached about the 18th to the 21st day from onset, but occurs much earlier than this after a single immunizing dose in an experimental animal. Thenceforward, in the absence of a relapse of the disease, or of a second immunizing injection, the curve falls, at first very steeply, then more and more gradually, till after the lapse of a few months, or it may be a year or more, the agglutinating power is almost wholly extinguished.

The power of conferring agglutinating properties upon the serum is not one equally resident in all species of bacteria. It is present in high degree in most members of the *B. coli* and typhoid group and in the cholera vibrio. It is somewhat less active in the case of the dysentery bacilli, and amongst the pyogenic cocci it is still weaker. But there are few, if any, pathogenic species in which 'agglutinogenic power' is altogether absent.

The phenomenon of agglutination is in a high degree a *specific* one in the sense that it is manifested chiefly against the species, or in some cases the serological race, which has evoked it and which is termed the homologous race or species. The word 'homologous' is also used in a still narrower sense for the actual strain which has been used in immunization. The power of agglutinating may not, however, be absolutely limited to the homologous species. An immune serum may also agglutinate heterologous species or races, though commonly to a very much lower degree: the titre for the homologous species is so much higher than that for other species that due care will prevent mistakes. Such agglutination of extraneous species is chiefly seen in nearly allied forms and is termed 'group agglutination' or 'coagglutination'. Its significance and possible explanation will be discussed later.

It has been occasionally reported that colon bacilli freshly isolated from the stools of a case of typhoid fever have been agglutinated by typhoid immune serum; similar observations have been made in bacillary dysentery. So far as has been observed the phenomenon is a temporary one, the property of being agglutinated by the heterologous serum vanishing after subculture for a longer or shorter period outside the body. The term 'para-agglutination'

has been used for those instances in which extraneous bacteria possess a property, which is apparently temporary, of being agglutinated by a specific immune serum when isolated from a case of the corresponding disease. Its possible occurrence must be a warning never to be content with agglutination alone in the diagnosis of a bacterium; it must be confirmed by observation of all the ordinary characters in cultural tests.

The agglutination of bacteria is not necessarily associated with the action of an immune serum. It may be brought about by chemical means, notably by the action of acids. This 'acid-agglutination' appears to depend upon the hydrogen-ion concentration present in the mixture, different bacterial species varying in the precise concentration of hydrogen-ions to which they react (Michaelis). In any case, even when the clumping is brought about by the action of a specific serum, the presence of an electrolyte such as sodium chloride is essential to the process.

(2) The Nature of Agglutination.

There is still much to learn about the mechanism of agglutination, but in the present state of our knowledge it would seem likely that the process is dependent upon a change in electrical charge. It is known that in a bacterial suspension in normal saline the bacteria usually bear a uniform and weak negative charge: the similarly charged particles repel each other as they are known to do in colloidal suspensions in the 'sol' phase, and thus the suspension remains uniform. Any influence which neutralizes the electric charge, such for example as hydrogen-ion concentration in acid agglutination, may bring about approximation and adhesion of the bacteria. Similarly precipitation occurs when suspensions of two oppositely charged colloids are mixed.

The rôle of the electrolyte in causing the flocculation of a bacterial suspension is thus reasonably explained, but the manner in which the immune serum acts is by no means clear, and it is the serum which confers the specific element upon the process. We personify its action by saying that the serum contains an 'agglutinin', and we assume an 'agglutinogen' in the bacterium which evokes the antibody, but this takes us no nearer to an explanation of the mode of action of the serum. The facts that we know are these. When bacteria are brought into contact with the corresponding immune serum, some sort of union occurs between the bacterial protein and the unknown substance in the serum which leads to agglutination; possibly this union is of the nature of adsorption. If now the mixture be centrifuged, the clear supernatant fluid is found to be robbed of its agglutinating properties. The bacterial sediment, after treatment with the serum, when washed and suspended in distilled water shows no agglutination, but on the addition of an electrolyte undergoes flocculation. We may conclude that the union of the bacteria with the agglutinin of the serum has in some way 'sensitized' them so that they become amenable to the flocculating action of the electrolyte, but we have at present little or no idea as to the nature of the mechanism by

which the sensitization is effected. It has been proved that 'sensitized' bacteria can be flocculated by much lower concentrations of an electrolyte than unsensitized ones. This 'two-phase' hypothesis as to the process of serum agglutination is due to Bordet, and is now generally accepted. The fact that a serum may be heated to 55°–60° C. with little loss of its agglutinating power proves that its action is quite independent of complement.

(3) The Physical Conditions influencing Agglutination.

(a) *Temperature.*

As in other physico-chemical processes the temperature influences the velocity of the agglutination reaction. It occurs more rapidly as the temperature rises, up to an optimum of about 55° C., above which the reaction is impaired and soon ceases. It will take place perfectly at room temperature, but requires a much longer time to become manifest: the incubator at 37° C. occupies an intermediate position. The time limit for significant agglutination thus varies according to the technique employed.

(b) *Gentle Motion.*

Gentle motion accelerates the process of flocculation. When watched with the naked eye on a slide it occurs more readily if the slide be inclined to and fro: the virtue of a water bath in which the tubes are immersed for half their length appears to lie in the convection currents which are set up.

(c) *Nature of the Bacterial Emulsion.*

Many workers prefer to use a living broth culture or a living saline suspension from a young agar culture. Dead bacteria are, however, as readily agglutinated as living ones, and in some respects are more convenient, as emulsions can be kept in stock and are safe to handle. Broth cultures or agar suspensions may be killed by phenol, chloroform, or formalin, and these disinfectants also preserve the emulsions, preventing the growth of adventitious bacteria or moulds. Or the bacteria may be killed by heating, care being taken that the temperature does not exceed 55° or at most 60° C., lest agglutinability be lost. There is evidence that suspensions thus heated may agglutinate more readily than unheated ones. The *density* of the bacterial emulsion is not a matter of indifference; the bacteria must not be in excess or agglutination may be obscured. For the microscopic method a broth culture is suitable, or an agar suspension of similar density; this will contain about 1,000 million organisms per c.c., giving, when mixed with an equal volume of diluted serum, a density of 500 million per c.c. For the macroscopic method the appropriate density varies with the end-point chosen: where complete sedimentation is taken as the end-point a fairly heavy emulsion of 1,000 to 2,000 million bacteria per c.c. is suitable, but when the finer shades of agglutination at the upper limit of the titre are to be determined a more dilute emulsion of about 250 million bacteria per c.c. is preferable. When, again, the rough and ready method

is used of observing agglutination on a slide with the naked eye, it is best to employ a very heavy suspension of some 10,000 millions per c.c.

(4) Other Factors influencing Agglutination.

There are a number of other factors which come into play in the process of agglutination, many of which have received no adequate explanation. The chief are as follows:

(a) *Spontaneous Agglutination.*

Certain strains of bacteria, whether in broth culture or saline suspension, are apt to undergo agglutination of their own accord, apart from the influence of any serum. For this reason a control observation in which the specific serum is replaced by normal saline, or better by normal serum, should always be introduced. This source of fallacy is usually betrayed, in a series of tubes in which gradually increasing serum dilutions are tested, by the absence of any gradation of the agglutination as the limit of the titre is approached. This difficulty will usually be overcome by diminishing the percentage of sodium chloride.

(b) *Inhibition Zones.*

It is no uncommon thing, especially in sera of very high titre, to find the agglutinating action absent in lower dilutions, becoming more evident as the dilution rises, till it reaches a maximum which is maintained up to a point near the limit of the titre. Thus, with a serum having a titre of 1 in 10,000 or 20,000 no effect may be seen at a dilution of 1 in 50 or 1 in 100. The cause of such 'zones of inhibition' or 'pro-agglutinoid zones', as they are sometimes called, is still imperfectly understood. Eisenberg and Volk showed that they were best marked in sera which had been heated, or long preserved at room temperature. The phenomenon has a close parallel in the flocculation of certain colloid suspensions in which excess of one or the other reacting substance inhibits precipitation, and is sufficiently common in agglutination work to form a pitfall for the unwary worker. It must never be assumed as certain that because no effect is seen in the low serum dilutions agglutinating power is absent. A series of dilutions ranging up to the full titre of the serum should always be employed for the identification of an unknown culture.

(c) *Variations in Agglutinability.*

Differences exist amongst bacterial species as to the readiness with which they agglutinate. For instance, at 55° C. the typhoid bacillus will show nearly maximum agglutination within 2 hours, dysentery bacilli in 4 to 5 hours, and the meningococcus in 24 hours. These differences must be borne in mind in working with different species.

Apart from this, individual strains of the same species may exhibit marked variations, ranging from those which are so agglutinable that the process occurs spontaneously, up to those which

refuse to agglutinate even with a potent serum prepared by injection of the strain itself into an animal, although such a serum may readily agglutinate other strains. The cause of this variation is still uncertain, but in some cases at least it seems correlated with variations in virulence. Many spontaneously agglutinating strains are ones which have been kept long under cultivation and have lost their virulence; conversely, inagglutinable strains are commonly ones which have just been isolated from the body, and such strains after a few subcultures may become agglutinable. It has been observed that strains which have been subjected to animal passage have suffered concomitant diminution of agglutinability. It has therefore been suggested that the inagglutinable condition represents one in which the bacterium is able to resist the action of antibodies, much as virulent bacteria may be insusceptible of phagocytosis. While this explanation may cover part of the truth it does not cover all, as old cultures kept long on solid media may also be deficient in agglutinability, though this is usually restored by passage through broth. The method of daily subculture in broth for a week or two has a marked effect in promoting agglutinability, and no strain should be pronounced inagglutinable till this expedient has been tried.

Broth cultures appear more stable in their agglutinating properties than suspensions of agar cultures, especially when the latter have been heated. It is a difficult thing to prepare, after even a short interval, two emulsions from one strain which will give identical readings with the same serum, and the divergencies may be very considerable. In a reaction so complex as that of agglutination it is impossible to reproduce all the factors concerned in an experiment with absolute exactness. This is one reason for preferring emulsions killed and preserved in bulk, in place of newly made suspensions, where a series of comparative observations is being made. It is this principle which has been adopted and extended by Dreyer in preparing the standardized emulsions issued from the Oxford laboratories, each issue of which is compared with one of known agglutinability so that a factor can be assigned to it, which enables comparable results to be obtained with different emulsions.

(d) *Group Agglutinins.*

Allusion has already been made to the fact that heterologous bacteria are often agglutinated by a specific serum, though usually to a much lower titre than the homologous organism. Instances have indeed been recorded in which the titre for the heterologous has been actually higher than that for the homologous organism.

Group agglutination is commonly regarded in actual practice merely as a troublesome pitfall, and its phenomena are apt to be dismissed as of little account. Nevertheless its study may reveal facts of considerable importance as regards the antigenic structure of bacteria. Group agglutinins are not mere accidents, and the view now usually held as to their significance is as follows. The correspondence between an agglutinin and the antigen which evokes it during immunization is admittedly specific, i. e. the

agglutinin can only sensitize the particular protein concerned in its evocation. There must be some slight structural differences between different agglutinins which prevent their affecting any but the protein with which they correspond. When therefore we find a specific serum, i.e. one prepared with a single bacterial strain, capable of affecting two distinct bacterial species, or races, though perhaps to a differing degree, we can only explain it by one, or both, of two hypotheses. Either the serum must contain two agglutinins which must have been produced by the stimulus of two antigens, both present in the species used in immunization, or, if only one agglutinin is present, the two bacterial species affected must contain an antigenic component in common. Either hypothesis leads to the same eventual conclusion, that more than one antigen is present in the bacterial body, and we may go on to infer that the difference in the titre of the serum for the two species is governed by the relative abundance of the different antigens in each respectively. An extension of this argument leads to the doctrine of 'multiple antigenic components', first explicitly formulated by Durham. This teaching regards the bacterial protoplasm not as a homogeneous entity, but as a mosaic of slightly differing proteins, each capable of evoking its own specific antibody. But in each race or species there is usually a marked predominance of one particular antigen, and it is this which confers the stamp of individuality. The serum to which such a bacterium gives rise contains a corresponding excess of the 'specific' agglutinin, while the minor 'group' agglutinins are due to the remaining antigenic components. On this hypothesis coagglutination implies the existence, in the bacteria involved, of some common antigenic constituent, and may thus afford a clue to bacterial relationships. It is natural that the serological races of a single species should exhibit a greater wealth of components in common than is the case with distinct species, and in fact we find that such species as the meningococcus and Flexner's dysentery bacillus present a high degree of group agglutination amongst their various races.

While group agglutination is thus of theoretical importance, in routine practical work it may lead the beginner into error. It is needful always to know the specific titre of the serum with which one is working, by the actual technique employed, and to beware of attaching undue importance to an agglutination which reaches only to a small fraction of this titre. It is claimed that group agglutination is least troublesome when formalinized broth cultures, as advocated by Dreyer, are employed.

(e) *Natural Agglutinins.*

The serum of a normal adult man or animal may possess some degree of agglutinating power upon a bacterium, although the individual in question may never have been known to suffer from the disease which it causes, nor have been knowingly immunized against it. Since this phenomenon seems absent in the newborn, it must be an acquired property, and it is conjectured that it is due to a 'sub-infection', perhaps not infrequent, in which pathogenic bacteria gain temporary access to the body in insufficient

numbers to cause disease. It is of interest, in this connexion, to note that the serum of a laboratory worker who has for some time been engaged in the study of a given bacterium, has occasionally been found to possess an agglutinating power above the normal for that particular organism.

The liability to the development of agglutinins by this natural process is of importance in practical work. The average titre observed is fortunately a low one, but it has to be determined in the case of each common pathogenic organism, so that we may be in a position to avoid drawing diagnostic conclusions from agglutinations manifested below a certain degree of serum dilution. The point below which agglutination may be deemed of no practical significance varies in the case of different bacteria, perhaps in some relation with the commonness of each amongst any particular population. In the case of the typhoid bacillus a titre below 1 in 25 or 1 in 30 is held of no diagnostic significance, although in the case of the hitherto much less common *B. paratyphosus A* (which further possesses much less agglutinogenic power in man than *B. typhosus*) a titre of 1 in 10 or even less is believed by some to be significant. In this country, at least, a titre of 1 in 25 against Shiga's dysentery bacillus has a probable diagnostic value, whereas at least double this titre should be demanded before a patient can be held to have been recently infected with the much more common Flexner group of dysentery bacilli. In testing a patient's serum it is the rule to start in each case at the lowest dilution which may be regarded as significant, with the technique employed, and work upwards from this.

(f) *Multiple Agglutinins.*

Cases in which two or more primary specific agglutinins are simultaneously present in a serum must be distinguished from those of mere group agglutination. When a person suffers from two infections at the same time, or one after the other, the production of antibodies against each proceeds just as if the other were not present. If an animal be immunized with a dose of bacterium *A* and a week later with a dose of bacterium *B*, the agglutination curve of each pursues its own course, the curve for *A* reaching its maximum a week earlier than that for *B*. Only where there is marked racial affinity between the two species does the dose of *B* produce any further rise in the *A* curve. The presence of multiple agglutinins has of late derived practical importance from the introduction of the triple vaccine against the enteric group. The effect of this vaccine is to evoke, in the serum of the inoculated person, agglutinins against all the three bacilli employed —*B. typhosus* and *B. paratyphosus A* and *B*, the titre for typhoid being the highest and that for paratyphoid *A* the lowest, since the three organisms are not equal in agglutinogenic power. The serum usually manifests these agglutinins for a year or more after inoculation. If, now, such an inoculated person develops either of the three diseases while his blood still contains the agglutinins due to his inoculation, the problem of sero-diagnosis becomes difficult. The single observation which suffices in an uninoculated

person no longer gives sufficient information, but a presumptive diagnosis in a large number of cases can be made by taking the titre of the serum against all three organisms or three or more successive occasions spread over a week or so. The curve for one of the three organisms will commonly be found to follow that to be expected in the corresponding disease, while the other two curves either remain level or show a 'sympathetic' rise less in degree and sometimes earlier in date than in the case of the first. It is not indeed always possible to interpret the results correctly, and it must be admitted that the diagnosis requires not only a good technique but considerable experience, while at times, and notably when the disease occurs immediately after inoculation, it may be frankly impossible by this method.

II. The Technique of Agglutination.

The methods which may be employed for determining the fact of agglutination and for measuring the titre of a serum fall under three headings. In the *microscopic* method the clumping of the individual bacteria is watched under a high power of the microscope. In the *macroscopic* method the process is carried out in small test-tubes and the flocculation is watched with the naked eye. There are further a number of *intermediate* methods, mostly designed for rapid clinical or laboratory diagnosis. These methods will now be described in detail, but the greater importance of the macroscopic method has led the committee to deal with it more fully than with the others.

(1) The Microscopic Method.

The microscopic has one indisputable advantage over the macroscopic method; it can be carried out with very minute quantities of serum, even with less than a drop. It is also more speedy—from half an hour to an hour being all that is required. Against these advantages must be set the grave disadvantage of quantitative inaccuracy. It is impossible to carry out a series of dilutions of a minute quantity of serum with the same exactness as can be attained with a larger volume, while the end-point of the reaction is very ill-defined. For this reason, in spite of the fact that the method requires no other apparatus than an ordinary microscope, this method cannot be recommended for routine use. Though at one time widely used, it is now almost universally abandoned for accurate quantitative work. Nevertheless it still has its value when the amount of available serum is too small for the more accurate macroscopic method or where a merely qualitative result is required.

The technique is as follows. The *bacterial suspension* usually employed is a living 24 hour broth culture or a suspension in normal saline either from a 24 hour agar culture, or, even better, from a gelatine culture not more than four or five days old. The suspension must not be too dilute: it should be visibly turbid. It should be examined microscopically before use, to ascertain that it

is free from accidental clumps. Killed cultures may be used, but this method does not lend itself well to the employment of the standard agglutinable emulsions which have proved of such value in the macroscopic method.

A very accurate method of carrying out the dilutions of small quantities of serum is undoubtedly by the aid of a haemocytometer pipette—a 1 in 10 dilution being made with the white counter and a 1 in 100 with the red, using normal saline as the diluent. From these further dilutions can be prepared.

A simple, though perhaps not very accurate, method of preparing a series of dilutions from a very small quantity of serum is that employed by Wright and described in his 'Technique of the Teat and Capillary Glass Tube', London, 1912. It is shortly as follows:

Take a simple capillary pipette, or better, a throttled pipette (see Fig. 7), furnished with a rubber teat, and make a mark on the stem some 2 or 3 cm. from the distal end. Holding the pipette so that the teat may be compressed between the finger and thumb, expel about half the contained air and introduce the end of the capillary tube into the serum. Cautiously relaxing the pressure on the teat, allow the fluid to run up into the tube as far as the mark and then check the inflow. Now remove the pipette from the fluid and, again cautiously relaxing the pressure, admit a short column of air some $1\frac{1}{2}$ to 2 mm. in length. The end of the pipette is next introduced into a watch glass or other receptacle containing normal saline and a column of the diluent allowed to reach the mark. As many units of diluent, separated by air bubbles, are taken up as will suffice to produce the required dilution: thus if a dilution of 1 in 10 is wanted, the single unit of serum is followed by 9 such units of saline. The contents of the pipette are finally expelled into a watch glass, or on to a glass slide, and well mixed by reaspirating into the pipette several times. By a repetition of the process, the 1 in 10 dilution can be converted into a 1 in 100, and this into a 1 in 1,000, or up to any desired point.

The next step is to mix equal volumes of the bacterial emulsion and the different serum dilutions, a process which is best carried out with a capillary pipette provided with a suitable fiducial mark on the stem. Some trust to a platinum loop for transferring equal volumes to a glass slide, but this method cannot be recommended. Many workers prefer to put up the mixtures as 'hanging drops'[1], but there is little or no advantage in this procedure. The reaction can be observed perfectly well on a plain glass slide, with an ordinary cover glass. If a somewhat thicker layer of fluid be desired the

[1] A hanging drop is prepared as follows: Sterilize a perfectly clean cover glass by passing it once or twice through the flame of a Bunsen burner. When cool, place a platinum loopful of a just prepared mixture of diluted serum and bacterial emulsion in the centre of the cover glass. Have in readiness a 'hollow ground slide' (one, that is, with a shallow circular depression ground in its centre) painted with vaseline round the edges of the hollow Lower the slide, upside down, on to the cover glass, so that the drop comes in the centre of the hollow on the slide. Turn the slide the right way up, and by pressure with a pair of forceps secure a perfect sealing of the cell by the vaseline. Any air channel left will lead to rapid drying of the drop. In examining with the microscope, close down the diaphragm, find the edge of the drop with a low power, and then focus with a higher power.

corners of the cover glass may be held, in turn, in the by-pass flame of a Bunsen burner, so as to produce a minute thickening on each. In any case the edges of the preparation must be sealed with vaseline to prevent evaporation. It is desirable to put up a control slide with normal serum. A $\frac{1}{6}$ in. objective is the most suitable for examination since it yields a wider field of view than a $\frac{1}{12}$ in. oil immersion, which gives a needlessly high magnification.

The slides should be examined immediately they are put up, and then at intervals of some 15 minutes for an hour. Agglutination will take place at room temperature, but it is better to keep the slides in an incubator at 37° C. If no clumping occurs in an hour the result is to be regarded as negative: trivial aggregations of only two or three bacteria are best disregarded. If the result be positive, the highest dilution in which unmistakable agglutination has occurred within the time limit imposed is taken to mark the titre of the serum, but this end-point, as has already been remarked, will not be found very sharply defined, while the titre obtained is commonly much lower than that found by the macroscopic method.

(2) Intermediate Methods.

The methods which fall under this heading are for the most part rapid ones designed to save time and trouble. They are probably equal in value to the microscopic method, if not superior, but inferior in accuracy to the macroscopic method. Their chief field of usefulness is found in rapid clinical diagnosis, and especially in laboratory work in times of stress when very large numbers of plate cultures have to be examined within a short time.

(a) *Macroscopic Agglutination on a Slide.*

This is a useful rough and ready method for the determination of the nature of a bacterium but must be employed with due precautions. Two points must be kept in mind. (1) The serum must be neither so concentrated as to manifest group agglutination, nor so dilute as to require long for its action to be apparent. The precise dilution needed depends upon the titre of the serum, but for a serum with a titre of 1 in 3,000 or 4,000 a suitable dilution is 1 in 100. (2) The bacterial emulsion must be a heavy one, at least equivalent to 5,000 or 10,000 million organisms per c.c., or the clumping may not be readily visible to the naked eye. An ordinary broth culture is thus unsuitable, but a thick suspension may be made in normal saline either from a colony on a plate culture, or preferably from an agar subculture 24 hours old.

The *modus operandi* is exceedingly simple. Having prepared a homogenous emulsion of suitable density from a colony or culture of the unknown organism to be tested, such high potency sera are selected as are judged likely to react with the suspected bacterium. A large drop of a one-hundredfold dilution of each of these sera is placed in series on a slide and a drop of the emulsion added to each drop of the serum. If the organism belongs to species included in the sera selected, that drop exhibits visible granular agglutination in from 30 seconds to two minutes, the remaining drops acting as

controls. Clumping is hastened by gently rocking the slide to and fro about a longitudinal axis. A grease pencil mark between adjacent drops prevents their accidental admixture. Special long slips of glass may be employed when many sera are used. In place of single sera some workers employ mixtures of sera, e.g. of paratyphoid *A* and *B*, or of Shiga and Flexner sera, a process which saves time if the results are negative, but not when positive, as the agglutination must then be repeated with single sera. *In any case it must be understood that this rapid method is merely one for preliminary orientation, and may be wholly misleading; it cannot replace the cultural tests which are requisite for establishing the nature of a bacterium. Neglect of this principle may easily lead to serious errors in diagnosis.* The method is also applicable in the reverse direction, for the serological diagnosis of infectious disease, as was shown in the case of typhoid fever by Bass and Watkins.[1] For this purpose dense formalinized or phenolated emulsions of a series of appropriate bacteria are required, and may be kept in stock. A row of drops of such emulsions having been set out on a slide, a drop of the patient's serum, diluted to 1 in 10 or 1 in 15, is added to each, and the agglutination carried out as above. The emulsion which is earliest and best clumped is held to represent the infection from which the patient is suffering, but it is clear that much caution is requisite in accepting the results of such a rough and ready method as final.

(b) *Garrow's Agglutinometer.*

The apparatus devised by Garrow[2] is based upon the preceding method, but elaborated in such a manner as to give a measure of the actual titre of a serum to several bacteria at once. It consists of a porcelain slab containing rows of cups in which serum dilutions can be carried out, a series of bottles containing heavy emulsions of the appropriate species of bacteria, standard platinum loops, and a long slide on which the agglutinations are carried out. When the slide is charged with the drops of mixtures it is placed in a celluloid cylinder, the air in which is kept saturated with moisture; the cylinder is caused to rotate slowly by means of a clockwork drum attached. After five minutes of this constant movement the slide is removed and the resulting agglutinations read with a pocket lens. For fuller particulars reference must be made to Garrow's paper, which is clearly illustrated. Those who have used this apparatus speak well of it as a rapid and sufficiently accurate means of serodiagnosis; it is claimed that ten sera can be examined in an hour. As regards accuracy it is claimed that the readings are on the average within one dilution of that found by the full macroscopic method, but it must be remembered that this represents a possible 100 per cent. error. It is therefore plain that the field of usefulness of this apparatus lies where rapid work is required under stress of circumstances.

[1] *Arch. Int. Med.*, 1910, 7, 717.
[2] *Lancet*, 1917, i. 262.

(c) *Wright's Pipette Method*

This method is a rapid and convenient process for clinical work. It demands no other apparatus than a throttled capillary pipette with a long stem actuated by a rubber teat. A 'throttled pipette' is one in which sufficient air resistance is introduced to permit of easy and complete control over the movement of the fluid in the stem. A convenient form is shown in Fig. 7, and is made in the following manner. The barrel and stem are made separately and then luted together with sealing-wax. The barrel is made by nicking and breaking off a simple capillary pipette at the point where the neck passes out into the capillary stem. The stem is a piece of wide capillary tubing, 20 to 25 cm. long, made in the ordinary way by drawing out a wide glass tube in the blowpipe. The stouter end of such a capillary tube is drawn out in the by-pass of a Bunsen burner into a hair-like end, which is then broken off at its extremity so as to leave an extremely minute aperture to constitute the throttle. A drop of sealing-wax is now put round the stem so as to form a collar just below the commencement of the throttle. The open end of the stem is passed into the wide end of the barrel and pushed through until the collar of the other end is arrested in the neck of the barrel. By gentle warmth the sealing-wax is now melted and an air-tight junction thus formed between the barrel and stem, which must be kept in a straight line until cool. Finally a rubber teat is fitted on to the upper end of the barrel.



Fig. 7

In preparing a series of serum dilutions a mark is made on the stem from 1 to 2 cm. from its extremity, and successive unit volumes of saline are taken up, separated by air bubbles, the number corresponding with that of the dilutions intended, with the addition of one extra volume, because the first volume taken up is so largely wasted in wetting the pipette that it should be discarded. The unit volumes are then expelled as separate drops on to a clean glass slide or slides. One unit volume of the serum or dilution of serum with which it is proposed to start is next taken up into the pipette and added to the first drop, and well mixed by sucking backwards and forwards into the stem. A unit volume of this mixture is added to the second drop and similarly mixed, and so on with the successive drops, one volume of the last drop being discarded. One volume of pure saline is left at the end of the series as a control.

The pipette is now well washed out with saline, and as many unit volumes of bacterial suspension, separated by air bubbles, are taken up into the stem as there are drops on the slide, including the control. These volumes of bacterial suspension are now added, one to each drop on the slide. The mixtures are next taken up again into the stem, in suitable approximately equal volumes, separated by air bubbles, beginning with the control and ending with the strongest dilution of the

serum. The distal end of the tube is sealed in the flame, and the throttle closed with a drop of melted sealing-wax on a fine glass rod passed down the barrel. Care must have been taken at all stages to avoid the passage of fluid into the throttle, or it becomes blocked. If a control serum is needed, the same series of procedures is required with this also.

The tube, when charged and sealed, is placed upright at any desired temperature: it is convenient to stick it into a small tray of plasticine. The results are read after the appointed time, which will vary according to the temperature and the nature of the bacterium: they may be read with the naked eye, or by the aid of a hand lens.

(d) *Max Neisser's Method.*[1]

This method has been widely used in Germany and to some extent in this country. The agglutinations are carried out in small glass cells ground flat at the top and bottom, so that they can be piled in series: small flat salt-cellars can be used if nothing else is available. In these the dilutions of serum are carried out, either with a graduated pipette, as Neisser himself recommended, or by the drop method with calibrated pipettes: only two or three drops of fluid are needed in each cell. A series of cells having been laid out, in number corresponding to the dilutions to be examined with an extra one to serve as a control, one drop or volume of saline is placed in each. A similar drop or volume of an 8 or 10-fold dilution of the serum is added to the first cell, one drop or volume of this mixture to the second, and so down the series. Finally, a drop or volume of bacterial emulsion is added to each cell, after which the cells are piled up and the uppermost covered with a glass plate bearing the label. They are now placed for two hours in the incubator at 37° C. The results are read under a low power of the microscope ($\frac{2}{3}$ in. objective), each cell in turn being placed on the stage. Even a small degree of flocculation is apparent and the end point is sharply defined.

As compared with the ordinary microscopic method, Neisser's technique gives sharper and more trustworthy results. The titres obtained seem higher than those yielded by the macroscopic method. The great advantage offered by Neisser's method lies in the fact that large numbers of sera can be examined by its means in a short time and with reasonable accuracy. Rajchman and Western found that they could examine no less than 130 sera against 5 different organisms in a working day of 5 hours—a result which can scarcely be paralleled by any other technique. It may be doubted however whether, for exact quantitative work, it is equal to the macroscopic method: one obvious criticism is that there is danger of inaccuracy in the dilution of such small volumes of fluid, especially in open dishes liable to evaporation.

(3) The Macroscopic Method.

For routine purposes of clinical or bacterial diagnosis any of the methods so far described may be deemed adequate. It is

[1] Proescher, *Centralbl. f. Bakteriol* [etc.] 2. Abt. Orig. 1902 31, 400.

usually sufficient for such purposes that the fact of agglutination with a reasonable dilution of the serum should be established, without the need for absolute exactness in the determination of the titre. When however exact research work is attempted, for example even such a task as the plotting of the agglutinin curve after a single immunizing dose of bacteria in an experimental animal, most if not all of the preceding methods betray their lack of accuracy. There is a general consensus of opinion amongst those who have worked much at the subject that the macroscopic method is by far the best for exact research work, and is capable of refinements which permit of a higher degree of trustworthiness than any other method of agglutination.

While it involves a little more trouble and demands more apparatus than most of the preceding methods, it can be carried out with reasonable speed, indeed as quickly as the microscopic test. It is a disadvantage that it requires a larger volume of serum than other methods, preferably one cubic centimetre if the reaction has to be carried out against several bacteria; the withdrawal of this amount by syringe from a vein is now, however, a routine pathological practice, and indeed the requisite amount can be obtained without much trouble from the finger. *The Committee therefore recommend that, whenever possible, some form of the macroscopic method be employed in agglutination tests.* Two forms of technique will be fully described—the one in common use and the more delicate one described by Dreyer. But before this is done it may be of service to discuss certain details which have a bearing on all macroscopic methods, and are indeed vital to the highest accuracy.

(a) *The Bacterial Suspension.*

The bacterial suspension employed may be in the form of a broth culture or of a saline emulsion of a young agar culture, and may be living or dead. A living broth culture is usually a sensitive and satisfactory suspension with which to work: in the case of an organism refractory to agglutination, repeated subculture in broth is sometimes successful in overcoming the difficulty. Dreyer prefers veal broth for this purpose, and claims that by daily subculture in this for two or three weeks the agglutinating power of the organism tends to become increased and stabilized. Living agar suspensions in normal saline may be used, but must be diluted to standard opacity; even broth cultures vary in turbidity, and when comparable results are required with a series of organisms this source of error should be corrected, for the density of the suspension employed has a marked effect upon the apparent titre yielded by a given serum. Suspensions made from agar cultures may be advantageously somewhat thicker than broth cultures.

Actual counting of the bacteria per c.cm. is for most purposes an unnecessary refinement; it is usually sufficient to judge the degree of turbidity by the naked eye, using good artificial illumination in a darkened room. In selecting a standard opacity regard must be paid to the nature of the end-point chosen. If this is to be complete sedimentation on standing, a higher degree of opacity

may be used than where 'standard agglutination' just visible to the naked eye is selected as the end-point. The finer degrees of agglutination towards the limit of the titre are best judged with a thinner suspension, and one corresponding to 250 to 300 million per c.cm. may be aimed at. In general terms the thinner the suspension, within reasonable limits, the higher is the titre disclosed in the serum. For the grosser end-point offered by complete sedimentation one of about 1,000 million per c.cm. serves well. In order to secure uniformity of working it is desirable to make permanent tubes which can be preserved as a standard. For this purpose suspensions of barium sulphate in 1 per cent. sodium citrate solution have been recommended. These can be made of the degree of turbidity which the worker has found best adapted to his purpose, and all bacterial suspensions can be diluted to this standard before use.

Dead bacteria are agglutinated as well as living ones, and there are two advantages in their employment. They entail less risk to the worker, and emulsions can be prepared in bulk and stored, after addition of a preservative, thus effecting not only a saving of time but greater constancy of results. Two methods are in use for the preservation of bacterial emulsions: in one the organisms are killed by heating, in the other by the action of chemical agents.

An agar suspension in saline which has been heated to 55° C. for half to one hour, usually agglutinates more rapidly than a living one, but many are of opinion that it does not yield such constant results. Broth cultures may similarly be sterilized by heat. In either case, if the emulsion is to be preserved for long, an antiseptic such as 0·5 per cent. phenol should be added.

Killing by the agency of chemicals is on the whole preferable to heating. Phenol, chloroform, or formalin may be employed, but of these formalin has proved the best, probably because it hardens and fixes the bacteria in such a manner that autolysis does not occur later, as is liable to happen with most other preservatives. The required strength of commercial formalin (40 per cent. formaldehyde) is 0·1 per cent., and the only defect of this method of preservation is that it is insufficient to check the growth of moulds: considerable care must therefore be taken to avoid accidental contamination when the bottles are opened. Broth cultures of dysentery, though not of typhoid bacilli, thus formalinized, undergo at first a considerable diminution in agglutinability, but after the lapse of 2 to 3 months they become stable and thenceforward remain practically constant. The method of preparing standard agglutinable emulsions will be more fully described later in discussing Dreyer's technique.

One further point remains to be mentioned in connexion with bacterial emulsions and it is one of greatest importance in all comparative work. No matter whether the emulsion is a broth culture or a suspension from agar, whether it is living or dead, whether it has been killed by heat or chemicals, there is no guarantee that it is equal in agglutinability to another emulsion prepared in the same way from the same stock culture. The difference in the titre of a serum apparently revealed by two suspensions which have been derived within a short time from the same

strain may amount at times to upwards of 100 per cent. Different strains of the same bacterial species or race may differ enormously in agglutinability, and an individual strain kept long in culture is liable to exhibit similar variations. In ordinary laboratory work, where a single agglutination is done for purposes of diagnosis, this fundamental fact is not noticed, nor is it of any great importance. But in research work, involving comparisons of agglutinating power, where, for example, a single strain is repeatedly used as a control, the variation at once forces itself upon the attention. Unless this source of error is recognized mistaken conclusions may readily be drawn. Only one method of overcoming the difficulty has so far been devised: it is that introduced by Dreyer, of standardizing the agglutinability of each batch of bacterial emulsion prepared, in the manner which will later be described in detail.

(b) *The Preparation of Serum Dilutions.*

There are many bacteriologists who regard the making of a series of graduated dilutions of a fluid as a simple and almost automatic process, and it is probable that few have ever been at the pains to check the accuracy of the method they employ. It is nevertheless a matter of considerable difficulty to prepare an accurate series of doubling dilutions, starting from a relatively small quantity of fluid such as the amount of serum commonly available for an agglutination test, and too little heed is paid to the errors which may creep in. The simplest way to convince oneself of the reality and magnitude of the fallacies which may be introduced is to prepare a series of dilutions of some easily titrated substance such as calcium chloride, and check the results by chemical methods. In a series of dilutions an error in each step of the process is liable to become cumulative.

Two methods are available for making dilutions. In the first, use is made of some form of graduated pipette, by which the requisite volumes of fluid are measured out. In the second, measurement is by drops in which, other things being equal, the governing principle is surface tension.

(i) *Measurement by Pipette.* Assuming a pipette to be accurately graduated there are, nevertheless, certain liabilities to error inherent in its use. The chief are wetting of the inside of the instrument and accidental addition of fluid adhering to its outer side. The narrower the pipette and the smaller the volume of the fluid measured, the greater is the proportional error thus introduced. Thus careful weighings will show that after filling an ordinary 5 c.c. pipette with water and emptying it, about one per cent. of the fluid remains adherent to its inner surface; with a fairly stout capillary pipette the fluid left behind amounts to 3 or 4 per cent. of the total volume.

The danger of error from drops adherent to the outside of the nozzle must have struck every one who has worked with capillary pipettes. Such drops should be removed with filter paper before the contents are delivered.

In their volumetric work, chemists use 'delivery pipettes' in

which allowance is made for such sources of error. They are careful to dry their pipettes after use, or to wash them out several times with the fluid next to be measured. It is to be feared that many bacteriologists are less scrupulous.

If a series of doubling dilutions of a strong calcium chloride solution be carried out with a 5 c.c. pipette, up to 6 or 8 dilutions, and the results checked by careful titration against silver nitrate, it will be found that great care must have been exercised if the end product is not to differ by 5 or 10 per cent. from its expected value. An error of this order of magnitude is almost unavoidable by the pipette method as ordinarily employed, and it is probable that where measurements are carried out with a capillary pipette the error greatly exceeds 10 per cent.

There are certain rules by the observance of which the errors attendant on the pipette method of carrying out serum dilutions may be minimized. Having fixed upon the volume unit to be measured, which must depend on the amount of the serum available but which should be as large as possible, a series of such volumes of saline should first be measured into each of a row of small test tubes or watch glasses, before touching the serum itself. The pipette having been dried,[1] or washed out with the fluid to be tested, a unit volume of serum, or of the low dilution of serum from which it is proposed to start, is added to the first tube and thoroughly mixed with the saline by taking it up into the pipette and expelling it several times. A unit volume of this mixture is now added to the saline in the second tube and the mixing repeated, and so on down the series. The advantage of this procedure is that there is no alternate wetting of the pipette with serum dilution and water, which is the most fertile source of inaccuracy; the pipette is only wet with the mixture next to be measured out. The series of dilutions may be prepared in the actual tubes in which the agglutinations are to be carried out, but greater accuracy is insured by making them on a larger scale and transferring the requisite small volume of each to the agglutination tubes.

In serological work the use of calibrated pipettes affords a rapid and, when due precautions are observed, a reasonably accurate method of preparing dilutions and of measuring fixed quantities of reagents. It is probable that where considerable volumes require to be measured some workers will find less difficulty in using such pipettes than in counting without error a long series of drops. Therefore the practical details which must be attended to in employing pipettes will be described in some detail.

Sizes of Pipettes. It is convenient to use two sizes with a total content respectively of 0·1 c.c. and 1·0 c.c. The column of fluid must not be too short. Both kinds of pipettes are graduated to the orifice.[2]

[1] This may be rapidly done by washing out with distilled water followed by acetone.

[2] The following are convenient measurements for such pipettes: (1) 0·1 c.c. pipette graduated to 0·002 c.c.: external diameter $\frac{1}{16}$ in.; the length of the column of fluid corresponding to 0·1 c.c. is $6\frac{1}{8}$ in. The pipette tapers for the last $\frac{3}{4}$ in. and the external diameter at the orifice is $\frac{3}{64}$ in. (2) 1 c.c. pipette graduated to 0·01 c.c.: external diameter $\frac{1}{4}$ in.; length of column of fluid corresponding to 1 c.c. = $8\frac{1}{2}$ in.; external diameter at orifice $\frac{1}{16}$ in.

The 1·0 c.c. pipette is used for amounts greater than 0·1 c.c. and quantities differing by 0·05 c.c. may be measured; the 0·1 c.c. pipette is for smaller amounts, and quantities differing by 0·005 c.c. may be delivered.

It is, of course, important that the pipettes should be accurately calibrated, and this applies especially when several pipettes are employed in the same series of operations.

Method of use. For use the pipette may be actuated by an indiarubber teat, but some workers prefer a mouthpiece of plain glass quill tube (No. 5), the free end being cut across transversely and smoothed in the Bunsen flame, and the other end slightly drawn out so as to slip readily on to a convenient length of not too slender indiarubber tubing to which the pipette is attached. To obviate the danger of sucking up material into the mouth, a small piece of cotton wool may be packed loosely into one end of the indiarubber tube, but this should be changed when it becomes moist. When the pipette is being used the mouthpiece is held between the teeth and the junction between the glass stem of the pipette and the attached indiarubber tube is held between the thumb and the first and second fingers of the right hand. In order to deliver a measured amount, say 0·05 c.c., with the fine pipette, the following manipulations are required: (1) draw up fluid to slightly above the 0·05 mark, taking care that only the extreme end of the pipette is immersed in the fluid, then apply the tip of the tongue to the orifice of the mouthpiece, so as to close it, and continue to keep the tongue in this position until operation (4) is completed; (2) withdraw the nozzle of the pipette from the fluid, and bring it against the side of the vessel *above* the level of the fluid; (3) while maintaining the nozzle continuously against the vessel wall as described under (2), make slight pressure on the rubber with the fingers and thumb of the right hand until the level of the fluid in the pipette reaches the 0·05 mark; then cease making any further pressure; (4) insert the pipette into the vessel into which the fluid is to be delivered (if the amount to be delivered is very small, e. g. 0·01 c.c., pass the nozzle down to within a short distance of the fluid in the tube) and (5) blow out vigorously—while blowing, bring the nozzle into contact with the side of the tube above the level of the contents. (The object of bringing the nozzle against the vessel wall in (3) and (5) is to ensure that the fluid shall drain away from the pipette and that a drop shall not remain at the nozzle, which would cause a serious error in the measurement of the amount delivered.)

The pipettes need not be dried between successive operations; but they should be well rinsed by drawing up and rejecting normal saline three or four times between each operation. Each new reagent should then be drawn up several times into the pipette before beginning to measure out quantities. Where there is a danger that a trace of any reagent might have a powerful action, the pipette, after its albuminous contents have been well washed out, should be boiled for a minute or so in the sterilizing bath and *then allowed to cool thoroughly before further use.* (Great trouble may be caused by allowing serum to evaporate or coagulate in the pipettes: they are then best cleared by passing fine wire through

the nozzle and along the stem; in addition, they may be soaked in nitric acid.)

The following experiment, made by one of us along with Dr. E. L. Kennaway, shows that the pipettes in question can be used with sufficient accuracy in the sense that repeated measurements show very satisfactory agreement:

Strong HCl pipetted into 10 c.c. water and titrated with approximately $\frac{N}{300}$ NaOH.

Series I.

20 measurements of 0·01 c.c. HCl with 0·1 c.c. pipette.

Results of Titrations.

c.c. $\frac{N}{300}$ NaOH required for neutralization

2·50	once
2·55	twice
2·60	8 times
2·65	6 times
2·70	twice
2·75	once
	20 measurements.

Mean of Results = 2·623 c.c.
Mode „ „ = 2·60 c.c.
Extreme range of variation from mean = ± 4·8 per cent.
(16 of the 20 measurements differ from the mode by less than 2 per cent.)

Series II.

18 measurements of 0·03 c.c. HCl with the same pipette as in Series I.

Results of Titrations.

c.c. $\frac{N}{300}$ NaOH required for neutralization

7·70	3 times
7·75	once
7·80	6 times
7·85	twice
7·90	3 times
8·00	once
8·05	once
8·15	once
	18 measurements.

Mean of Results = 7·848 c.c.
(Mean of 0·01 c.c. measurements in Series I × 3 = 7·869, i.e. the difference from calculated mean is approximately 20 in 8,000, or 0·25 per cent.)

Mode of Results = 7·80 c.c. (i.e. exactly thrice the mode of the 0·01 c.c. measurements).

Extreme range of variation from mean = ± 2·9 per cent. (15 of the 18 measurements differ from the mode by less than 1·3 per cent.)

(ii) *The Drop Method.* The sources of error which are liable to attend measurement by pipette are entirely avoided by the drop method, but in their place we meet with others which demand careful consideration. The method rests upon the physical fact that successive drops of a given fluid issuing at constant rate from a clean pipette in a vertical position are precisely equal in volume. The difficulties which beset it lie in the maintenance of constant conditions.

We owe to Donald the elaboration of a technique which puts us in a position to make use of the drop method with accuracy, and his papers should be consulted.[1] Given the necessary care in the preliminary observations, there is no difficulty in preparing a series of dilutions which rival in accuracy the most careful measurements by pipette, and in which the error should not exceed 1 or 2 per cent. Further, in those cases where dilution by pipette is most liable to error, viz. in the measurement of very small volumes, the drop method maintains its accuracy: it is therefore peculiarly adapted for serological work.

The factors which govern the size of a drop, falling from a vertical clean pipette are chiefly two: (a) the *external* diameter of the nozzle of the pipette, and (b) the surface tension of the fluid. Subsidiary factors are (c) the rate at which the successive drops are formed and fall, and (d) temperature. Differences in laboratory temperatures are commonly too slight to be of appreciable influence, but the first three factors must be considered in detail.

Diameter of pipette. The internal diameter is of no consequence: it is the external diameter which governs the size of the drop, or, to be more accurate, the circumference of the tube at the contact edge where the drop clings round it before becoming detached. *It is for this reason that the pipette must always be vertical*, for any deviation converts the circle of contact into an oval of greater circumference, so that the drop becomes larger in proportion to the deviation from the upright position. Further any local greasiness of one side of the nozzle deforms the circle of contact and causes irregular results: the pipette must be absolutely clean and grease-free. Wide variations in the size of the drop can be achieved by varying the diameter of the nozzle, from drops of 5 c.mm. up to those of 50 c.mm.: with specially constructed nozzles Donald has been able to produce drops of water $\frac{1}{4}$ c.c. in volume.

He has shown that it is easy to produce pipettes of standard diameter by the use of an ordinary drill or wire gauge: such a gauge, which can be purchased at a good tool shop, consists of a steel plate perforated by numbered circular holes of graduated size (see Fig. 8). The freshly drawn capillary pipette is pushed into a suitable hole till it is arrested and is then cut off square with a glass knife at the upper surface of the plate. A fresh pipette of exactly equal diameter can at any time be made by repeating the process in the same hole. The pipette should become arrested some way along its stem, where it is nearly cylindrical: if it be cut off high up where it is rapidly tapering, the level of detachment of the drop, which may be 1 mm. or

[1] Ref. *Proc. Roy. Soc.*, Lond., 1913, **B. 86**, 198. *Lancet*, Lond., 1915, ii. 1243; 1916, ii. 423.

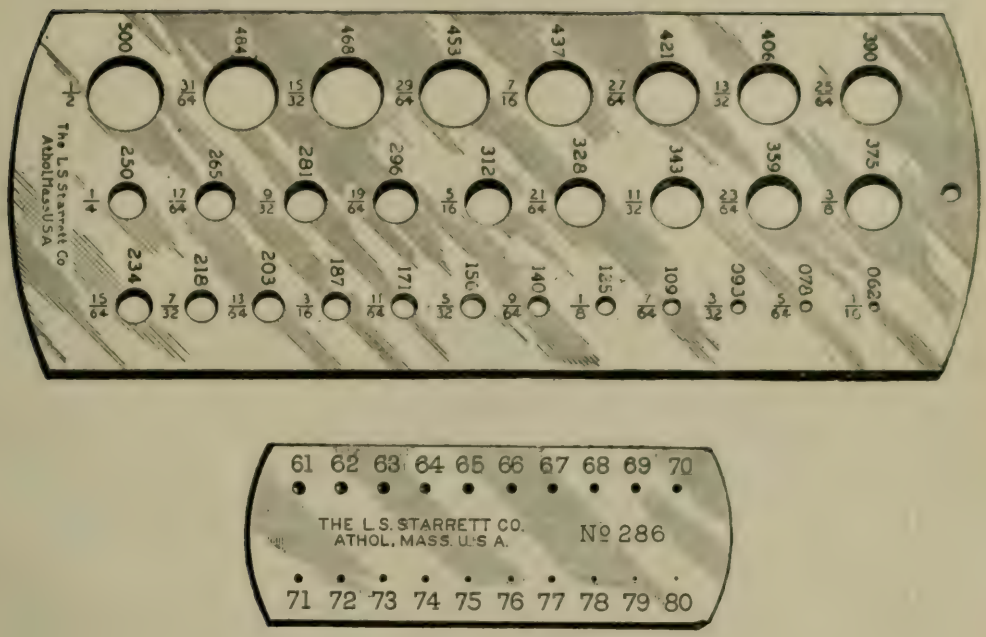


Fig. 8 Drill and Wire Gauges.



Fig. 9. Measuring bottle with file mark on neck for measuring drop volume.



Fig. 10. Dreyer's Agglutination Tube.
Total length, 5·5 cm.
External Diameter, max. 7·5 mm.
,, ,, min. 6·5 mm.
Internal Diameter, max. 6·0 mm.
,, ,, min. 5·5 mm.

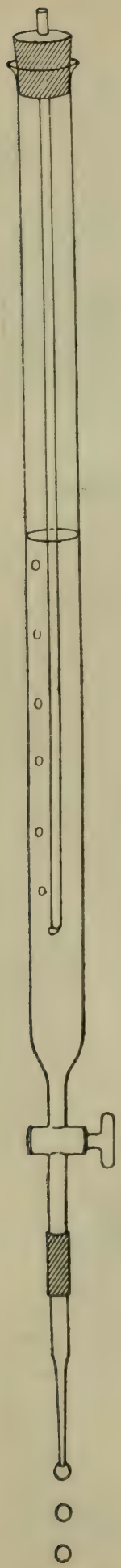


Fig. 11. Dropping Burette arranged with Mariotte's tube for dropping at constant rate.



Fig. 12.

Rubber teat, for use with Hand Dropping Pipette, exact size.



Fig. 13.

Fig. 13.

Hand Dropping Pipette.

Measurements.

Total Length, 19 cm.

Shaped upper-end to occupy not more than 1·5 to 1·8 c.m.

Lower end capillary of stout thermometer-tubing, 2·8 cm. long.

This leaves a *shaft-length* of 14·4 to 14·7 cm.

Thickness and bore.

Lower capillary end.

External diameter, maximum, 2·41 mm.
,, ,, minimum, 2·36 mm.
Bore, 0·5 mm.

Shaft.

External diameter, maximum, 5·4 mm.
,, ,, minimum, 5·0 mm.
Bore, 3·2 to 3·4 mm.

Dropping capacity.

When distilled water at room-temperature is delivered drop by drop by means of gentle, even pressure on the teat (the pipette being held *vertical* and being quite clean and free from grease), *25 drops* must have a volume of not more than 0·99 c.cm., and not less than 0·95 c.cm.

more from the tip, will not correspond with the hole in the gauge. The gauge and the glass knife must be kept free from grease, and the nozzle of the pipette must not touch the finger or any greasy object. Accidental traces of grease can usually be removed by immersing the nozzle in strong sulphuric acid and then washing with distilled water. Uniform pipettes are so easy to make that it is best to make a freshly drawn clean one as needed, rather than to attempt to clean one which has been used. The pipette can be furnished with a rubber teat for free-hand dropping, or, if very many drops of the same fluid are to be counted, it can be attached by a short length of rubber tubing to a burette or separating funnel, provided with a stop-cock.

The surface tension of the fluid. This exercises a very definite influence upon the size of the drop and has to be estimated for each fluid measured, so that allowance can be made for it. This can easily be done by ascertaining the relative number of drops, at constant rate from the same pipette, which are required to make up a known volume of fluid. Donald recommends small measuring bottles (see Fig. 9), holding 1 or 2 c.c., which can be made from a short length of wide glass tubing, sealed at one end and drawn out at the other into a neck of about 4 mm. internal diameter. Above the neck the orifice should be expanded. A transverse file-mark on the neck serves as a fiducial point. The neck must not be less than 4 mm. in diameter or the drops jam. The number of drops of water at constant rate required to fill the bottle up to the mark is now determined and checked once or twice. The pipette is then dried, and the process repeated with the fluid to be tested; the relation between the number of drops of the fluid and of water respectively required to fill the bottle allows of a determination of the relative size of the drop in each case. Alternatively the pipette can be attached to a burette filled first with water and then with the fluid to be tested, and, again being careful to keep the drop rate constant, the number of drops corresponding to 5 or 10 c.c. of each fluid can be ascertained. It is to be observed that this trouble need be taken only once. When the relative drop volumes of the different fluids to be measured have been ascertained the information is available for all future work. Donald[1] gives a list of the drop values of certain fluids commonly used in serological work as follows (the figures represent the relation between the number of drops required to fill unit volume, i.e. the smaller the figure the larger the drop):

Water	1·0
Saline	1·0
Fresh human serum	1·1
Inactivated rabbit serum	1·09
Guinea-pig serum	1·06
Cerebro-spinal fluid	1·02
Peptone broth	1·2
Standard agglutinable typhoid culture	1·2
Alcoholic antigen (in Wassermann test)	2·5

[1] *Lancet*, 1916, ii, 423.

These figures represent the particular samples tested by Donald, but clearly cannot be taken as absolute because different samples of the same fluid vary slightly in composition and hence in surface tension. Thus samples of peptone broth or standard agglutinable culture may be met with having a drop value of 1·1 instead of 1·2.

Since we are here concerned with the dilution of serum, it must be observed that, while different sera vary somewhat in their surface tension, a drop of serum is always so much smaller than one of saline that it is never safe to use one pipette for dropping them without correction. Donald's figure shows that 11 drops of fresh human serum are equivalent to 10 of water, but rabbit sera, preserved with phenol, may show a greater disparity than this. The drop factor of such sera may be between 1·2 and 1·3; thus, taking it at 1·25, 5 drops of serum would be equivalent to 4 of water. Such substances as phenol and thymol have a marked effect in lowering the surface tension of a fluid. Similarly, in jaundice, the bile in the serum lowers the surface tension and diminishes the size of the drop. The addition of serum to water does not much alter the surface tension of the latter till it forms more than 10 per cent. of the total. A 1 in 100 or 1 in 1,000 dilution of serum in saline has practically the same drop value as pure saline, and even a 1 in 10 dilution presents a quite negligible difference.

Rate of dropping. This may make a difference of nearly 10 per cent. in the size of the drops. A rapid rate increases their size. Donald has shown that the best results are obtained at a drop rate of 1 per second; if it is increased beyond this the size of the drops quickly rises, counting is less easy, and a constant rate is less easily maintained in free-hand dropping. A slower rate introduces less inaccuracy, but is unnecessary and tedious. Very little practice is requisite for regular dropping at about 1 per second with the free hand.

Constancy in the drop rate from a burette is best secured by introducing a 'Mariotte's tube'. This is a narrow glass tube, open at each end, passing through an air-tight rubber cork in the mouth of the burette, and reaching down to within an inch or two from the bottom. As the level of the fluid falls, air bubbles in and keeps the pressure above constant. (See Fig. 11.)

(iii) *The Preparation of Serum Dilutions by the Drop Method.* This may be done in either of two ways: (*a*) by the use of two calibrated pipettes delivering equal drops of serum and saline respectively, or (*b*) by using a single pipette, modifying the number of drops of each fluid so as to correct the error arising from their inequality in surface tension. The first of these methods is decidedly the better, since it avoids troublesome calculation as well as the difficulty that fractions of a drop cannot be measured with accuracy.

The only trouble attending the first or 'two pipette' method lies in the initial calibration of the pipettes, but with the aid of a gauge this can be overcome once for all. It is necessary to find by trial which two holes in the gauge will yield pipettes delivering sensibly equal drops of the two fluids. The numbers of these holes

can then be registered for all future use, and pipettes freshly prepared from them as needed. The error should not exceed 1 per cent.

With the 'single pipette' method the difficulty arising from fractions of a drop can be sufficiently overcome by a slight modification in the sequence of dilutions employed, shifting them from an exact geometrical progression to a series which will fit into the necessary scheme of drops.

Having in view all the considerations which have been set forth in the preceding section, most of which have been confirmed by a long series of experiments carried out by one of its number, the Committee are of opinion that, while reasonably accurate results can be attained by pipette measurements in the carrying out of serum dilutions, when a sufficient amount of serum (not less than 1 c.c.) is available, results fully as accurate can be attained by the drop method, and that where the volume of serum available is small the drop method possesses undoubted superiority.

Whatever method be chosen for making the serum dilutions, the most convenient series to adopt is a geometrical progression in which each dilution is double the preceding one. The starting-point must be the lowest dilution in which significance attaches to a positive result. Thus, in examining the serum of a patient suspected of typhoid fever, one would begin with a dilution of 1 in 20 or 1 in 25; if paratyphoid *A* were in question one might start at 1 in 10, whereas with a dysentery due to one of the Flexner group 1 in 50 would be a suitable starting-point. In calculating the serum dilutions required it will of course be borne in mind that the bacterial emulsion has still to be added. Thus, if an equal volume of emulsion is to be added and the series is to start at 1 in 20, the actual serum dilutions must start at 1 in 10; if $1\frac{1}{2}$ volumes of emulsion are to be added to 1 volume of serum dilution, and the latter starts at 1 in 10, the finished series will commence at 1 in 25. In all cases it is desirable to add a control tube, with normal saline or normal serum suitably diluted.

The only drawback to a series of dilutions in geometrical progression is that when, in the comparison between different sera or different emulsions the results are written down in columns, differences appear less than they really are. A difference of one tube in the end-point looks very little on paper, but in such a progression it is a difference of 100 per cent. In order to get a true view the results should be plotted out as a graph based on arithmetical progression, when the facts elicited may take on a different complexion.

In all accurate work it is important to push the series of dilutions to the end-point of the reaction; if this has not been attained at the first attempt the agglutinations must be repeated with a higher series of dilutions. Where the reaction is being carried out to determine the nature of a bacterium by testing it against known sera, it is important to know the titre of each serum by the technique employed, and to judge the results in terms of this. The significant thing is not so much the actual dilution

up to which agglutination occurs, for this will vary with the potency of the serum, as the *percentage* of its titre to which the serum agglutinates the unknown bacterium. Little diagnostic importance may attach to a reaction which only reaches $\frac{1}{10}$ of the full titre: even $\frac{1}{4}$ of the full titre may be of doubtful significance, owing to group agglutination, but $\frac{1}{2}$ titre and upwards may usually be taken as reliable.

(c) *The Dimensions of the Tubes.*

The dimensions of the tubes in which macroscopic agglutinations are carried out may exercise an appreciable influence on the end-point obtained. As a general rule it may be laid down that the narrower the diameter of the tube the better and more quickly is the agglutination brought about, so that the highest readings are obtained in capillary tubes. For ordinary purposes a convenient size of tube is one having a length of about 5 cm. and an internal diameter of 5 to 6 mm. Larger tubes, such as those used in the Wassermann reaction, may be employed, but somewhat lower readings will then be obtained, and the finer differences towards the end-point of the reaction will be less easily perceived.

In all comparative agglutination work it is essential to use tubes of uniform size. In a batch of purchased tubes some will usually be found narrower or wider than the rest, and these should be rejected. The results of different workers are only comparable when they have used similar tubes: it is therefore desirable that some standard size should be adopted for macroscopic agglutination, and this has been attempted during the war in the outfits supplied for use in the technique advocated by Dreyer. The Committee recommend the general adoption of this form of tube, which has a convenient flange at the orifice, and is pointed at the lower end so as to fit into a small hole in the supporting rack. (See Fig. 10.)

The racks used may be of any shape or size dictated by convenience, and each worker can have them made to suit the type of work on which he is engaged, since no principle is involved. For use at room temperature or at 37° C. wooden racks are suitable: for a water-bath or a temperature of 55° racks made of sheet zinc are usually employed.

(d) *Temperature and Time of Incubation.*

The temperature and time. of incubation must be considered together, since they are mutually dependent. At room temperature not less than 24 hours must be allowed for the completion of the reaction, and even in the incubator, at 37° C., the end-point may not be reached unless the tubes are left 6 or 8 hours, or even over night. This renders it desirable, or even necessary, to employ killed cultures for agglutination, for otherwise the reaction may be obscured by growth of the organism.

For several reasons a temperature of 50° to 55° C. is preferable to the lower temperatures just mentioned. Either killed or living cultures may be used as the worker prefers, though living cultures will not long survive at 55° C. The chief advantage lies in the shortening of the period required for a complete reaction, for the optimum

temperature for flocculation lies between 50° and 55° C. Either a hot air chamber or a water-bath may be used. In the former case it is well to place a dish of water in the chamber in order to minimize evaporation from the tubes. A water-bath is better: the immersed tubes more quickly reach the desired temperature, and if care be taken that the level of the water is only halfway up the columns of fluid in the tubes, the continuous convection currents set up produce that gentle movement which best promotes flocculation.[1] Comparative experiments show that in a water-bath the end-point is reached sooner than in a hot air chamber. The form of water-bath best adapted for agglutination work is a rectangular copper one, 2½ to 3 inches in depth, and of an area corresponding with the number and size of the racks to be employed: it is heated below by a gas flame controlled by a Hearson's or other form of regulator, and provided with a thermometer. To prevent precipitation on the tubes the water bath should be filled with distilled water.

The time required for a complete reaction varies with the nature of the agglutinable organism. At 55° C. the typhoid and paratyphoid bacilli are well agglutinated in 1½ to 2 hours, and little further change occurs on longer exposure. Dysentery bacilli require from 4 to 6 hours, and the action continues to progress slightly up to 18 or 24 hours: certain bacilli allied to the Flexner group must always be given 24 hours, and the meningococcus, which is usually agglutinated in 24 hours, may at times require as long as 48 hours. When the appointed time has elapsed for the reaction and the racks are removed from the water bath or hot chamber, it is desirable to leave them standing at room temperature before taking the readings, in order that there may be time for sedimentation to take place. The readings are thus rendered easier. Tubes from the water bath must be carefully wiped before reading.

(e) *The End-Point of the Reaction.*

When agglutination has progressed to its limit and the tubes have been allowed to stand a short time, it will be found that up to a certain point the flocculated organisms have sunk to the bottom of the tube leaving a perfectly clear fluid above. By many workers the upper limit of this complete sedimentation is chosen as the end-point, and this practice has the merit of offering a clearly marked and easily read limit to the reaction. It does not, however, indicate the real limits of the titre, for beyond it lesser degrees of agglutination will be found, in which sedimentation is incomplete or absent, and the flocculi still floating in the fluid pass from those easily visible to the naked eye down to those so fine as to require a hand lens for recognition. A common practice in recording results is to indicate complete sedimentation by three crosses (+ + +), incomplete sedimentation by two (+ +), and flocculation still visible to the naked eye, but without sedimentation, by (+).

Another method of fixing the end-point and recording the results is that advocated by Dreyer. He points out that at least

[1] Topley and Platts, *Lancet*, 1918, i, 800.

eight or ten degrees of flocculation can be distinguished by the practised eye towards the limit of agglutination. The terminology he uses is as follows: Complete clarification of the fluid on standing is called 'total' agglutination (T); flocculation readily perceived by the naked eye, but without sedimentation, is called 'standard' agglutination (S), while barely visible flocculation is called trace (tr.). Intermediate degrees are indicated by the symbols T−, S+, S−, tr+, tr−, and even higher refinements have been introduced. The end-point taken is 'standard' agglutination. The difficulty that different workers might not agree in their conceptions as to this point has been overcome by the recent issue from the Oxford laboratories of permanent tubes of typhoid and dysentery bacilli in which the agglutination has been carried out in a gelatine medium which is then fixed with formalin.

The advantage claimed for this mode of reading and stating results is that finer and more accurate readings are obtainable, greater precision being thus given to the end-point of the titre. That this claim is not unjustified is shown by the fact that, by the use of standard formalinized emulsions and with this method of reading a regular and consistent curve of agglutination can be demonstrated in typhoid fever and similar infections—a very difficult or impossible thing to do by other methods.

It may happen that no tube in the series shows 'standard' agglutination: thus one tube may show T− and the next tr+. To meet such cases Dreyer has calculated and published a reduction table by which standard agglutination can be computed.

'The full table of readings, with their reduction values, is as follows:

Reduction Table.

Total	T	1·47
Total minus	T−	1·29
Standard plus	S+	1·13
Standard	S	1·00
Standard minus	S−	0·88
Trace plus	tr+	0·77
Trace	tr	0·68
Trace minus	tr−	0·60
Query trace	? tr	0·53
Nil	0	0·46

The great value of this table lies in the fact that it enables us to calculate the serum-quantity or dilution that will give standard agglutination although we have not actually a tube in the series which gives a "standard" reading, e. g., if a dilution of $\frac{1}{50}$ of a serum gives a reading of "tr+" by reference to the table, we can see that standard agglutination will be given by a dilution of

$$\frac{1}{50} \times \frac{1{\cdot}00}{0{\cdot}77} = \frac{1}{38{\cdot}5}.$$

This table, though calculated for the typhoid-coli group of bacilli, is fully applicable to dysentery-agglutination, provided that a considerably finer flocculation be taken as "standard agglutination" for the latter than for the former group of bacilli.'

When, in research work, a number of sera are being compared against a given bacterial emulsion, or a number of emulsions against a given serum, the comparison should be carried out as

originally suggested by Madsen by matching the exact degree of agglutination in tubes from different series: those which are found identical afford an index by which the comparison can be accurately made.

(f) *Illumination.*

The gross end-point offered by complete sedimentation is easily appreciated by daylight, but when a judgement has to be formed as to finer degrees of agglutination a good form of artificial illumination is indispensable. The best form is offered by an electric light with frosted globe, enclosed in a blackened box or otherwise shaded so that no direct rays reach the eye of the observer. The room should be darkened, and the tubes so held that they are fully illuminated against a dull black background.

For those with good vision naked eye readings are best: degrees of agglutination which cannot thus be perceived, with the illumination just mentioned, may be disregarded. For those with defective vision, and especially for those who have become presbyopic, optical assistance is necessary and ordinary reading spectacles may be insufficient. Some form of weak lens may be used, the weaker the better, but the worker who is compelled to resort to this must be careful to correlate his readings with those obtained by the naked vision of his more fortunate or younger colleagues.

(4) Two Forms of Technique for the Macroscopic Method.

The principles which underlie the macroscopic method of agglutination having now been set forth two forms of technique will be described: (*a*) the ordinary or unstandardized method to which most workers are accustomed, and (*b*) the standardized technique advocated by Dreyer.

(a) *The Ordinary Method.*

A series of tubes judged sufficient to cover the known or anticipated titre of the serum is arranged in a suitable stand. A dilution, e.g. tenfold, of the serum is made, and starting from this a series of doubling dilutions with saline. Unit volumes of the tenfold and succeeding dilutions are placed one in each of the tubes and to each tube is added a unit volume of the bacterial emulsion. A tube at the beginning or end of the series is reserved as a control, receiving saline and emulsion only. The contents of the tubes must now be well mixed either by inversion of the tubes, thorough stirring with a platinum wire, aspiration with a pipette, or by bubbling air through the mixture by means of a fine pipette. The dilutions, which will now run 1 in 20, 1 in 40, &c., must be indicated on the tubes or stand. The stand is now placed in a water-bath or incubator at the desired temperature and left for the number of hours known to be required for the agglutination of the bacterium concerned. It is then removed and left to stand for 15 or 20 minutes at room temperature. Readings are finally taken under suitable illumination.

The choice of methods in preparing the serum dilutions and the bacterial emulsion, and also of time of incubation and end-point, will

be found fully detailed in the preceding paragraphs and need not be recapitulated here. The Committee regard this ordinary method of macroscopic agglutination as adequate for routine laboratory work. It suffices for the confirmation of the nature of an unknown bacterium by means of known sera, and for the establishment of a diagnosis in suspected disease by means of a known bacterial emulsion, provided that due care is taken to observe the principles which have been laid down in the foregoing pages. For the most refined comparative work, however, such as may be required in an elaborate research, or in following the precise titre of the blood from day to day, it is excelled in accuracy by the method next to be described.

(b) *Dreyer's Method.*

The technique with which Dreyer's name is associated is based in part upon a choice of the best features in older methods, and in part upon a principle which is novel and peculiar to it. Amongst the former Dreyer employs the drop method for all measurements, thin formalinized broth cultures for the bacterial emulsion, a water-bath at 55° C. for incubation, and a defined degree of agglutination close to the limit of the serum's titre as the end-point. These features combine to render the technique as delicate and sensitive as possible. When they are fully carried out they constitute a 'standard' method of carrying out agglutination which probably represents the best obtainable technique. Dreyer, however, has gone further than this in endeavouring to produce standardized bacterial emulsions of known and constant agglutinability, with the object of avoiding the known inconstancy of casually prepared emulsions. Thus, he has laid down a principle which, if established, enables us to estimate the agglutinating power of a serum in definite, if arbitrary 'units', and it is this which forms the novel and most valuable element in his method.

The possibility of establishing and maintaining a standard of agglutinability might at first sight be doubted when both the standard bacterial emulsion and the serum by which its agglutinability is tested are liable to change. It is, however, well established that such changes, though at first rapid and not inconsiderable in the case of certain organisms, become, after a few months' storage in the dark at low temperature, so slight and slow that both emulsion and serum may for practical purposes be regarded as stable for at least a year and even for two years. This cannot be proved for a single emulsion, for it is plain that if both emulsion and serum altered at the same rate, but in opposite directions, considerable loss of agglutinability would escape detection. The proof has therefore to be obtained indirectly. A given emulsion *A*, stabilized by storage, is chosen as an arbitrary standard, and is tested as regards agglutinability with a standard serum, and an arbitrary coefficient of agglutinability is assigned to the emulsion. After an interval of, say, six months, a new emulsion *B* is prepared and stored till it is stable. The agglutinability of this is now tested against the standard serum, emulsion *A* being tested for comparison at the same time; a coefficient of agglutinability is thus assigned to

emulsion *B* and it may differ from that of *A*. After a further six months a third emulsion *C* is similarly tested, and later still emulsion *D*, each receiving its coefficient from a comparison with *A*. If now all four emulsions, tested against the standard serum, and corrected for differing intrinsic agglutinability by means of their coefficient, reveal the same titre in the serum. the stability of the emulsions is sufficiently demonstrated, for the emulsions stored respectively for 1½ years, 1 year, and 6 months behave in the same way as the one last prepared. It is inconceivable that changes, had they occurred, should so exactly balance one another in emulsions stored for such different periods, and it is impossible that any change in the serum should balance corresponding changes in all four emulsions simultaneously.

The principles underlying the establishment of the agglutinin unit and the history of their development have been set forth in a memorandum written, at the request of this committee, by Dr. A. Gardner, and printed as an appendix to this report. Standardization of bacterial emulsions and agglutinin unitage have been set up for *B. typhosus*, *B. paratyphosus A* and *B*, Shiga's bacillus, and certain strains of Flexner's bacillus. During the war these standard emulsions issued from the Oxford laboratories under the auspices of the Medical Research Council for use in the Army and Navy, and have been employed on so large a scale that there has been ample opportunity for putting them to the test. It should be clearly understood that they must be used on the principles laid down by Dreyer, and the results read and calculated in the prescribed manner, if uniform and comparable results are to be obtained.

The details of Dreyer's technique, both as regards the preparation and standardization of the emulsions and as regards the carrying out of the test, are best given in the form in which they are issued from the Oxford laboratories. The leaflets sent out with the emulsions are herewith reprinted as follows:

DIRECTIONS

Preparation and Standardization of Agglutinable Cultures.

B. typhosus, B. paratyphosus (A and B), B. dysenteriae (Flexner, Shiga, and Y), B. enteritidis (Gaertner), B. Coli, and Vib. Cholerae.

1. Preparation.

The bacillus is grown for 24 hours at 37° C. in ordinary *veal* peptone bouillon[1] in large Erlenmeyer flasks partly filled (1 litre of bouillon in a one and a half litre flask).

Before use the flasks of bouillon are sterilized in the autoclave at 115° C. for *not more* than 15 minutes, and are then tested for sterility by incubation at 37° C. for 48 to 72 hours.

They are inoculated with a few drops each from a 20 to 24 hours old bouillon culture of the bacillus (*B. typhosus*, *B. paratyphosus*, &c.).

The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.

[1] The bouillon is titrated against phenolphthalein, and two-thirds of that amount of sodium hydrate, which would render it neutral to phenolphthalein, is added before the final boiling and filtration.

At the end of 20 to 24 hours' growth at 37° C. the flasks are well shaken, and to each is added 0·1 per cent. (1 c.c. per litre) of commercial (40 per cent.) formalin. They are again shaken and placed in a *cold chamber* in the dark at about 2° C.

At intervals on the same day and on subsequent days for 4 or 5 days the flasks are again thoroughly shaken and *replaced at once in the cold chamber.*

After 3 or 4 days they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton-wool.

2. Standardization.

The process of standardization consists (*a*) in making up the killed culture to an *opacity* as nearly as possible identical with that of the Standard Agglutinable Culture, (*b*) in measuring its *agglutinability* as compared with the Standard Agglutinable Culture by the use of Standard Serum.

Dysentery cultures should after dilution to standard opacity be left to stand in the cold store for one or two months before they are standardized for agglutinability and used for agglutination tests.

(*a*) **The degree of opacity** (turbidity) of the new culture as compared with that of the standard culture is determined as follows:

Take two stands and place eleven agglutination tubes in each. The tubes must be chosen so as to be of very nearly equal internal diameter (for this purpose some kind of a gauge is almost essential), and their surface must be devoid of scratches.

Prepare

(1) a few c.c. of a 1 in 2 dilution of the new killed culture.
(2) ditto ditto of the standard culture.

With the dropping pipette (held vertically) measure out

	Drops of water.	Drops of culture.	
Into tube 1 of each stand	0	20	Culture full strength.
„ 2 „	4	16	
„ 3 „	8	12	
„ 4 „	10	10	
„ 5 „	12	8	
„ 6 „	6	14	Culture diluted 1 in 2.
„ 7 „	8	12	
„ 8 „	10	10	
„ 9 „	12	8	
„ 10 „	14	6	
„ 11 „	16	4	

One series of tubes receives the new-killed culture, the other the standard culture. Mix the fluids in the tubes well, and take comparative readings with artificial illumination against a dark background. A frosted electric bulb with a large opaque shade and a piece of dull black board or paper propped up behind gives the best results.

Hold up the tubes to be compared against the edge of the lamp-shade, which is arranged so that the source of light is just hidden from the eye, while it illuminates the particles suspended in the fluids. A darkened room renders the reading easier and more accurate. The outside of each tube must be cleansed and wiped dry before a reading is taken.

A tube is chosen at random from one series, and it is compared with the tubes of the other series in succession until one is found to match it in opacity. The identity of these two tubes is recorded as the first reading.

If the tube chosen fails to correspond exactly with a tube in the other series, but is seen to have an opacity intermediate between the opacities of two adjacent tubes, the reading is recorded thus:

e.g. Series I tube 3 = Series II tubes 4–5.

Several tubes in one series are thus matched in succession with tubes of the other series, so as to obtain a number of separate readings.

Suppose that two of the readings are:

	Standard culture series.		New culture series.
(1)	Tube 5	=	Tube 9.
(2)	Tubes 2–3	=	Tube 6.

(1) Reference to the table shows that tube 5 contains 8 drops of undiluted culture, tube 9 contains 8 drops of a 1 in 2 dilution, i. e. 4 drops of undiluted culture.

Therefore 4 drops of the new culture give the same opacity as 8 drops of standard culture, i. e. the new culture is twice as opaque as the standard.

(2) Tube 6 contains 14 drops of diluted or 7 drops of undiluted culture. Tube 6 in the new culture series falls midway between tubes 2 and 3 of the standard series which contain 16 and 12 drops of full strength culture respectively. It therefore corresponds with 14 drops of standard culture.

Again it is found that the new culture has twice the opacity of the standard.

In practice the readings are found to vary a few per cent. on either side of the mean, and therefore it is best to take at least 5 readings and calculate an average. From these data the degree of dilution necessary to bring the new culture to standard opacity is determined.

e. g. in the example quoted the new culture must be diluted one in two.

The diluting fluid used is normal saline solution to which 0·1 per cent. of commercial formalin has been added.

If a high degree of accuracy in the opacity-estimation is required, the measurements may be made with a graduated 2·0 c.c. pipette into larger tubes, tenths of a c.c. being substituted for drops.

(*b*) To measure the agglutinability of the killed culture thus diluted proceed as follows:

Take two stands[1] and place 12 agglutination tubes in each. Prepare (1) a dilution of Standard Agglutinating Serum of such strength that each cubic centimetre contains from 4 to 8 Standard Agglutinin Units, and from this prepare (2) a second dilution of half that strength.

With the pipette measure out

	Drops of Normal Saline Solution.	Serum dilution.	
Into tube 1 of each stand	0	10	drops of dilution 1.
,, 2 ,,	2	8	,,
,, 3 ,,	4	6	,,
,, 4 ,,	5	5	,,
,, 5 ,,	6	4	,,
—	—	—	
,, 6 ,,	3	7	drops of dilution 2.
,, 7 ,,	4	6	,,
,, 8 ,,	5	5	,,
,, 9 ,,	6	4	,,
,, 10 ,,	7	3	,,
,, 11 ,,	8	2	,,
,, 12 ,,	10	0	,,

To each tube of one stand is added 15 drops of *Standard Agglutinable Culture*, and to each tube of the other stand 15 drops of the *killed culture* under standardization.

At each stage of the procedure the pipette is carefully washed and dried out with successive quantities of absolute alcohol, followed by successive quantities of ether.

The stands are placed for 2 hours (in the case of Dysentery cultures 4½ hours) in a water-bath at 50° C. 55° C., then allowed to stand for 15 to 20 minutes at room temperature and a reading subsequently taken by selecting in the series made with Standard Agglutinable Culture the tube which exhibits Standard Agglutination (the highest dilution in which marked agglutination, without sedimentation, can be detected naked-eye), and ascertaining which tube in the other series shows the same degree of agglutination. This comparison is repeated and made more exact by selecting in succession from the first series one or two other tubes which exhibit less than Standard Agglutination and similarly matching them in the second series. Should the tube be the same in each series the agglutinability of the killed culture is clearly equal

[1] Stands, dropping pipettes, agglutination tubes, &c., can be obtained from Messrs. Baird & Tatlock, Hatton Garden, E.C., or from Messrs. R. B. Turner & Co., Eagle Street, Southampton Row, W.C.

to that of the Standard. If not the same, the degree of agglutinability of the killed culture is now readily determined.

Thus suppose that tube 5 in the Standard series corresponds to tube 2 in the other series. The Standard Agglutinable Culture is twice as agglutinable as the killed culture under standardization, since only half the quantity of serum has been required to agglutinate it to the same degree.

Hence, if any given serum presented for examination is found to agglutinate this particular killed culture in a dilution of, say, 1 in 500, then 500 multiplied by 2 and divided by the figure given on the label of the Standard Agglutinable Culture is the number of *Standard Agglutinin Units*[1] in 1 c.c. of the serum examined.

Or again, if the killed culture were, say, 1·3 times as agglutinable as the Standard Agglutinable Culture, then, in the same example as above, 500 divided by 1·3 and again divided by the figure given on the label of the Standard Agglutinabie Culture is the number of *Standard Agglutinin Units* in 1 c.c. of the serum examined.

In order to increase the accuracy of the standardization the test of sensitiveness to agglutination should be repeated *ab initio* two or three times, and an average taken of the readings thus obtained.

Readings should always be made by artificial light against a dark background.

From the Department of Pathology, University of Oxford, on behalf of the Medical Research Committee.

DIRECTIONS

Use of Standard Agglutinable Cultures in testing the Agglutinating Power of a Serum against Dysentery Bacilli.

1. Technique.

Take a stand[2] which will hold 15 agglutination tubes and place in it 2 or 3 rows of 5 each, as may be required, and a dilution tube.

With the proper dropping pipette (held vertically) measure out into the dilution tube 36 or 54 drops of normal saline solution, 0·85 per cent. sodium chloride in distilled water (where the water supply is pure, tap-water can be used instead of saline solution), by means of gentle pressure on the teat.

Wash out the pipette with distilled water.

Dry out the pipette with successive quantities of absolute alcohol, followed by successive quantities of ether, and drive off the ether completely.

Take up the serum to be tested into the dried pipette. Measure out 4 or 6 drops of the serum into the dilution tube already containing the 36 or 54 drops of saline solution, thus obtaining a dilution of 1 in 10. Mix thoroughly.

Carefully wash out the pipette.

With the pipette measure out into each row of tubes as follows:

Number of tube	Drops of Normal Saline Solution.	Drops of Serum Dilution 1 in 10.		
1	0	10	to each tube in row 1 add 15 drops of *B. dysenteriae Flexner* Standard Agglutinable Culture.	If a mark be made on the pipette corresponding to a volume of 15 drops the fluid may be added without drop-counting.
2	5	5		
3	8	2	to each tube in row 2 add 15 drops of *B. dysenteriae Shiga* Standard Agglutinable Culture.	
4	9	1		
5	10	0	to each tube in row 3 add 15 drops of *B. dysenteriae Y* Standard Agglutinable Culture.	

[1] The *Standard Agglutinin Unit* is that amount of agglutinating serum which when made up to 1 c.c. volume with normal saline solution causes Standard Agglutination on being mixed with 1·5 c.c. of a particular Standard Agglutinable Culture and maintained at 50° to 55° C. for 2 hours (Dysentery cultures 4½ hours) in a water-bath followed by 15 to 20 minutes at the room temperature.

[2] See note 1 on p. 131.

At each stage of the procedure the pipette is carefully washed with distilled water, and either dried out with successive quantities of absolute alcohol followed by successive quantities of ether, or where quantities suffice washed out once or twice with the next fluid to be taken in hand. **One and the same pipette must be used throughout the operation.**

Shake each tube thoroughly in order from right to left, i.e. beginning each row with the highest dilution.

Place the stand for 4½ hours in a water-bath at 50°–55° C. (*not* in dry air).

In Tube 1 of each row the serum acts in a dilution of 1 in 25.
" 2 " " " 1 in 50.
" 3 " " " 1 in 125.
" 4 " " " 1 in 250.

Tube 5 containing no serum is control against spontaneous agglutination.

If the limit of agglutination is not reached within this series higher dilutions are followed out in a similar manner.

Thus, for example, 57 drops of normal saline solution plus 3 drops of a 1 in 10 serum dilution will give a serum dilution of 1 in 200, and, using the same quantities as before, one has the serum acting in dilutions of 1 in 500, 1 in 1,000, 1 in 2,500, and one in 5,000. And similarly for higher dilutions.

The tubes are examined after 4½ hours at 50°–55° C. followed by 15 to 20 minutes standing at room temperature. The reading is taken by comparing each tube in succession with the control tube, and should if possible be made by means of artificial light against a black background. (If daylight is used, the tubes inspected should be partly shadowed by passing a finger up and down behind them.)

Note.—**An identical procedure is used in testing the agglutinating power of a serum against Standard Agglutinable Cultures of** *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. enteritidis* (**Gaertner**), *B. Coli*, **and** *Vib. cholerae*, **except that the tubes are only left in the water-bath for 2 hours**

It is advisable to re-examine the tubes after a further period of 16 to 20 hours at room temperature, when possible zones of inhibition will have ceased to complicate the readings. The readings will be found to have moved on slightly in the interval.

The highest dilution in which marked agglutination (without sedimentation) can be detected by the naked eye is *Standard Agglutination*. This in the case of the dysenteric bacilli is always much more finely granular with smaller flocculi than in the case of enteric (typhoid and paratyphoid) bacilli. Owing to the rate at which the dilution increases in the series of tubes employed, it will commonly happen that no tube in the series exhibits *Standard Agglutination*. If this be so it will be found in looking along the series that while one tube shows strong agglutination with sedimentation the next succeeding tube shows no agglutination at all or only a trace. In such cases Standard Agglutination lies approximately midway between the two dilutions.

Should a more precise determination of the limits of agglutination be required, it can be obtained by using a stand of 12 tubes with the series of quantities given in the table contained in the directions for Preparation and Standardization of Agglutinable Cultures.

(If the stand is left at the room temperature, 16 to 24 hours must be allowed before the reading is taken, but the reaction is not then so sharply defined. In this case the highest dilution in which a definite sedimentation with clearing of the supernatant fluid appears corresponds approximately to Standard Agglutination.)

When the standard degree of agglutination (*'Standard Agglutination'*) occurs with Standard Agglutinable Culture in a serum dilution of 1 in x, then x divided by the figure given on the label of the Standard Agglutinable Culture employed gives the number of *'Standard Agglutinin Units'* contained in 1 c.c. of the serum examined.

Thus if Standard Agglutination occurs in a dilution of 1 in 100 and the number on the label is 2, then $\frac{100}{2}$, i.e. 50, is the number of Standard Agglutinin Units contained in 1 c.c. of the serum examined.

For uniformity and simplicity in recording results they should be expressed in *Standard Agglutinin Units*.

I. **Diagnosis of Bacillary Dysentery** caused by *B. Flexner*, *B. Shiga*, or *B. Y*, by means of Standard Agglutinable Cultures.

A In persons who do not give a history of bacillary dysentery and who have not been inoculated against dysentery, the presence of 10 or more Standard Agglutinin Units per cubic centimetre of serum[1] is for all practical purposes diagnostic of an active bacillary dysenteric infection caused by the bacillus agglutinated. The presence of from 6 to 8 units[1] justifies a strong suspicion of dysenteric infection. In the latter case the test must be applied again in the course of a few days (4 or 5) to ascertain whether there is a definite change (rise or fall) in the agglutination titre.

B. In the case of persons who give a history of bacillary dysentery the serum may, even when years have elapsed since the infection, still contain 10 or more agglutinin units per cubic centimetre. In such cases it will, before a diagnosis can be made, be necessary to carry out a second examination. If such a second examination, conducted 4 or 5 days after the first, shows a definite change (rise or fall) in agglutination titre, we are dealing with an active infection. But if no important change in the agglutination titre is shown the agglutination is to be attributed to a past infection with bacillary dysentery.

C. A 'Carrier' in whom the infection is inactive will usually show no important change in his agglutination titre on repeated examinations at short intervals.

II. **Diagnosis of Bacillary Dysentery in cases of mixed infection** by two or more varieties of *B. dysenteriae.*

When in the course of a differential diagnosis it is found that the patient shows a high agglutination titre for two or more of the dysenteric bacilli, the question arises whether we are dealing with co-agglutination, or with agglutination persisting from a past infection with another form of bacillary dysentery, or with a mixed infection. In the two former cases it will be found that the rise and fall in agglutination to the different microbes will be synchronous. It is to be noted in connexion with co-agglutination that it is more common to see a co-agglutination of *B. Flexner* in a *B. Shiga* infection than of *B. Shiga* in a *B. Flexner* infection.

When the case is one of mixed infection the agglutinin curves for the different infecting organisms are usually not synchronous, and they pursue their ordinary course independently of each other.

From the Department of Pathology, University of Oxford, on behalf of the Medical Research Committee.

Sept. 6, 1916.

The Committee have carefully considered the evidence as regards Dreyer's method, both from the point of view of the principles on which it is based, and the practical results which have attended its use. While they have already expressed the opinion that the older methods of performing the macroscopic agglutination test are adequate when it is merely desired to ascertain the fact of agglutination of a bacterium by a known serum, or the rough titre of a serum against a specific bacillus, they recognize the fact that the use of standardized emulsions imparts to the test a degree of precision which is lacking in other methods. The principles involved in standardization appear to rest on good experimental evidence, and the consistent results which, in careful hands, have followed the application of the method in actual practice bear out the claims made for it. Dreyer's method, in the opinion of the Committee, constitutes a definite advance in the technique of agglutination, inasmuch as it seeks, with apparent success, to eliminate one of the most troublesome variables in the process, namely, the wide differences in the agglutinability of different emulsions of the same

[1] In the case of women the figures 10 units and 6 to 8 units should be replaced by 20 and 12 to 16 respectively, for the serum of normal women exhibits a higher agglutination titre than that of normal men.

organism. The method is therefore especially to be recommended in all agglutination work involving comparative results, since it appears to be the only one by which accurate comparisons are attainable.

If standardized emulsions are accepted and employed, a change in the terminology by which the results are expressed is a natural corollary. The end-point of the titre of a serum cannot logically be stated in terms of the dilution at which it is observed when this dilution varies with the individual emulsion employed. In order to express the result when the dilution is corrected by the coefficient of agglutinability of the emulsion, Dreyer has proposed an arbitrary 'agglutinin unit'. The definition of this unit and the reasoning by which it has been reached will be found in Dr. Gardner's memorandum in the Appendix. It must be realized that the system is applicable only to species which are serologically homogeneous; in those presenting distinct serological races it cannot be applied to the species as a whole, though it could, if thought desirable, be applied to each race severally. Further, the agglutinin unit is a convenient abstraction, determined separately for each species or race, and the units for each of these are not necessarily comparable amongst themselves. The general principle, however, which has been adopted in fixing the unit is to make ten units coincide, as far as possible, with the lower limit of significant agglutination in the diagnosis of the several diseases concerned. It is not pretended that the agglutinin unit has the same order of value as, for example, the gramme; if all the standardized emulsions of a given species were lost the unit for that species could not be exactly reproduced; a new one would have to be established; this is of course true also of the unit of diphtheria antitoxin.

Bearing all this in mind, and realizing that there may be other and unknown factors in agglutination for which no allowance or correction is at present possible, the Committee are nevertheless of opinion that this method of correcting variations in agglutinability by the adoption of an arbitrary agglutinin unit, in those cases to which it can be applied, is a step in the right direction. It is doubtless not a final step, but since it offers a more uniform method of expressing the titre of a serum than uncorrected dilution, it seems the most scientific method at present at our command and has the merit of conciseness. It is precisely by adopting a correcting factor of this kind that further advances will be facilitated.

So far then as the central feature of Dreyer's system is concerned, the Committee find that it has an apparently sound basis. Certain other and less essential details of the technique may also receive a few words of comment. The formalinized broth cultures sent out for use are diluted down to the minimum opacity consistent with clear readings, and the strains selected for making the cultures are chosen on account of their sensitiveness to agglutination. These features combine to render the reactions obtained very delicate, and it is therefore somewhat surprising to find that the phenomenon of group agglutination is far less conspicuous with this technique than with those previously used. The reason is not apparent, but the fact is attested by all who have worked with this method, and has

been found advantageous in avoiding the confusion in diagnosis which group agglutination sometimes causes.

The use of the drop method with a single pipette, for putting up the test, has been criticized on the ground that the different fluids mixed are of different surface tension and must yield unequal drops. This criticism has been met by Ainley Walker [1], who shows that when the prescribed number of drops are mixed, the inequalities in size so far balance each other that while no one of the tubes has exactly the supposed serum dilution, the error does not exceed 3 per cent. in any. It is to be observed that the use of the drop method is not essential to Dreyer's technique; the fluids can be measured in any desired way so long as the ratios of the ingredients, namely two parts of the serum dilution to three parts of the bacterial emulsion, are maintained. Further, it is of the greatest importance that the agglutination tubes should be of standard measurements, even slight divergencies of the internal diameter leading to fallacies. The tubes should also be scrupulously clean, new tubes should be washed first in acidulated water, then rinsed in large volumes of tap water, and finally in distilled water; they are then dried in an oven. Any tubes showing cloudiness of the walls after drying should be discarded. After being used the tubes should be cleaned by a similar process.

After the tubes have been filled in with the mixture of serum and bacterial emulsion they should be placed in a water bath, preferably containing distilled water, at 55° C. in such a manner that only from $\frac{1}{3}$ to $\frac{1}{4}$ of the tube is immersed in the water.

The first reading should be taken after the tubes have been in the bath for at least 2 hours in the case of the enteric group of bacilli and for at least $4\frac{1}{2}$ hours in the case of the Shiga and Flexner types of dysentery bacilli, and have stood on the bench for about 20 minutes at room temperature. The second reading, which should never be dispensed with in all comparative work, should be made after the tubes have stood overnight, 16 to 20 hours, at room temperature.

Allowing the tubes to remain in the water-bath for longer periods than those mentioned above is sometimes advantageous and may be necessary in exceptional cases, e. g. with certain members of the dysentery group or strains of bacilli having a slow reaction time.

In concluding their remarks on Dreyer's method, the Committee would point out that it practically demands some central laboratory whence standardized emulsions can be issued. It is true that a competent bacteriologist can make and standardize his own emulsions, and official directions are issued for doing this, but in practice few are likely to take the necessary trouble and fewer still to do it as accurately as it can be done by an experienced worker who has devoted much time to the method of standardization. During the war the Medical Research Committee have furnished free supplies to the Forces from the Oxford Standards Laboratories, and the Committee strongly recommend that supplies shall continue to be available at a moderate cost from this or some other central source.

[1] *Lancet*, 1916, ii, 552.

III. The Absorption of Agglutinins.

Although Bordet had shown that bacteria could absorb their appropriate agglutinins from a normal serum, the first to put agglutinin absorption to practical use was Castellani. The principles laid down by Castellani in 1902[1] have formed the basis of an important method of diagnosis: these principles are shortly as follows. When an animal is immunized against a given bacterium, such as *B. typhosus*, its serum not only acquires the property of agglutinating that organism, but often in much lesser degree that of agglutinating sundry related species also. The latter agglutinins are termed 'secondary' or 'group' agglutinins, or 'co-agglutinins' in opposition to the 'primary' or 'specific' agglutinin for the homologous organism. Castellani showed that when such an immune serum was saturated with a sufficient dose of the homologous bacillus the serum, when freed from the bacilli, had lost not only its primary but also its secondary agglutinins. When, on the other hand, it was saturated with one of the heterologous organisms for which a co-agglutinin had been developed, only that co-agglutinin was removed from the serum, the primary agglutinin being wholly or almost untouched. If an animal is simultaneously immunized against two different organisms, primary agglutinins against each are developed in the serum. Under these circumstances, Castellani showed that saturation of the serum with one of the two organisms removed its own agglutinin only, leaving that for the other practically intact.

These observations, which seem to imply some difference between a primary and a secondary agglutinin not yet wholly explained, furnish us with a valuable means of establishing the complete identity of two bacterial strains, or the reverse. The method is of importance in any attempt to divide up a species into serological races, for it holds good amongst these as well as amongst distinct species. It has indeed come to constitute a court of final appeal in all such cases.

Certain bacteria, e.g. the Aertrycke bacillus and *B. paratyphosus B* are so closely allied that the only possible way of distinguishing them is by the absorption test (Bainbridge). It is of course possible that the organisms are merely different races of the same species.

(1) The Technique of the Absorption Test.

The reaction is a quantitative one, and the amounts of serum and bacteria should be properly adjusted. The principles involved may be considered under several headings.

(a) *The dilution of the serum.*

The more dilute the serum the greater the relative amount of agglutinin absorbed, although the absolute amount absorbed is greater, up to a certain point, the less dilute the serum. Many workers employ a dilution of 1 in 50 or 1 in 100, and this practice

[1] *Zeitschr. f. Hyg. u. Infektionskrankh.*, Leipz., 1902, 40, 1.

has the advantage of enabling a large dose of bacteria to act upon a small amount of serum, so as to secure a maximum effect. It has, however, the obvious disadvantage, that when once the serum has been diluted to this point lower titres cannot be ascertained, and it remains uncertain whether or not the titre for a given organism has been reduced to virtual extinction. With a dilution of 1 in 10 this objection is largely overcome, and in actual practice an absorbing dose of almost any size can be used with this dilution of the serum; according to the titre of the serum a dilution of ten to one hundred fold may be recommended for routine work.

(b) *The bacterial emulsion.*

Most workers employ a living suspension of an agar culture in absorption work. Killed cultures, however, absorb agglutinin perfectly well, indeed cultures which have been heated to above 60° C. have been shown still to absorb agglutinin. It is probably of no very great consequence whether a living or a killed culture is used.

(c) *The absorbing dose.*

It is desirable that this should be known and recorded with accuracy, and it should be stated in relation to the volume of undiluted serum upon which it acts. The most convenient way of expressing the dose is in millions (of bacteria) per c.c. of undiluted serum. In a great deal of the absorption work which has been done in the past there has been considerable looseness in this respect. The worker has been content to add so many loopfuls of solid culture, or so many agar slopes, to his diluted serum: these procedures are lacking in scientific accuracy. It is easy to arrive at a fairly close approximation to the dose by counting with a haemocytometer, or by estimation of the dose by the opacity method. The actual emulsion employed will probably be too dense for either method, but a portion of it can be diluted down to suitable density. The dose required to effect adequate absorption varies in different groups of bacteria, and must be determined by trial. In general terms it may be said that, amongst the enteric group, doses of a million million per c.c. of undiluted serum will not be found excessive, though a quarter of such a dose will usually effect nearly all the absorption which can be achieved. In the case of dysentery bacilli, much smaller doses are requisite: even 1,000 million will usually bring about a definite reduction in titre, and a maximum effect is commonly reached with 25,000 to 50,000 million. Emulsions containing 50,000 to 100,000 millions per c.c. can readily be prepared by washing down four or five well-grown agar plates into 5 or 10 c.c. of saline. The quantitative nature of the reaction is well exhibited by exposing the serum to the action of a series of graduated doses, and if the results are plotted out graphically the titre of the serum is found to fall rapidly at first, then more and more slowly till it approaches zero. The last traces of agglutinin are difficult or impossible to remove, even in theory.

A useful plan for routine work is to employ a small and a moderately large dose in parallel experiments; where the titre of the serum against several organisms is being tested, the smaller dose usually brings out a selective action upon certain of these titres which may afford useful information. Should the larger dose produce a further fall, nearly to zero, in all the titres it may be concluded that the absorbing bacterium is homologous with the serum, i.e. identical with that used in immunization. If the larger dose produces no further fall in certain of the titres, though that for the absorbing organism is extinguished, it may be concluded that the organism is heterologous, i.e. not identical, or at least not completely identical, with that used in the preparation of the serum.

It is sometimes recommended to saturate the serum twice or more with the absorbing bacterium. It is doubtful whether this procedure is of much value, if an efficient absorbing dose has been employed in the first instance, and it is somewhat difficult to carry out without liability to error. If it is desired to repeat the saturation, a measured volume of the supernatant fluid remaining from the first operation should be mixed with an equal volume of fresh bacterial suspension containing the required second dose, and it must be noted that the serum dilution is now double that which it was at first. The practice is probably in any case inferior to that of graduated dosage in the first instance.

(d) *Temperature and duration of incubation.*

The fixation of agglutinin by the bacteria occurs during the preliminary phase of sensitization and is independent of subsequent flocculation, though the latter habitually follows when the conditions are suitable, and is of material assistance in the removal of the bacteria from the exhausted serum. Sensitization occurs quite readily at room temperature, and there is no need to place the mixture in the incubator. A period of 2 hours is quite sufficient for the fixation of the agglutinin, and during this time flocculi will probably have begun to separate out. Should it be desired to hasten the process of flocculation, the mixture may now be placed for awhile in the incubator at 37° or even at 55° C.

(e) *Sedimentation.*

Where agglutination is complete, sufficient sedimentation may occur naturally in the course of a few hours, or on standing overnight. More often the centrifuge is required to obtain a perfectly clear supernatant fluid: with good agglutination ten minutes in the centrifuge will suffice, but if no absorption has occurred, or if a gross overdose of bacteria has been used, many hours may be needed to clear the fluid sufficiently for testing.

(f) *Provision of a control.*

It is always needful, when putting up an absorption experiment, to reserve a tube as a control. This will contain serum alone, diluted with saline to the same degree as the tube or tubes containing the absorbing mixtures. This control tube must accompany

the other tubes through any vicissitudes of temperature and environment incidental to the experiment: it thus serves as the base line from which the effects of the absorption can be calculated.

(2) Description of an Absorption Test.

The general principles underlying the absorption method having now been discussed, a detailed description may be given of one good way of performing the test. The actual volumes used must depend upon the amount of serum available: here it is assumed that 2 or 3 c.c. are at hand.

On the previous day a sufficient number of agar slopes or plates will have been inoculated to yield the amount of emulsion required. Four to six agar slopes, or one large or two small plates, are as a rule enough. The growth, having been inspected for purity, is washed down into 5 or 6 c.c. of sterile saline, preferably freshly prepared. The emulsion will probably have a density of 10,000 to 20,000 million bacteria per c.c. Half a c.c. of the emulsion is now added to such a measured volume of saline as to render it of moderate turbidity only, and this dilution is either counted or its bacterial content estimated by the opacity method. The strength of the original emulsion can thus be computed, and it can be diluted down to 5,000 million per c.c. Two clean sterile centrifuge tubes are now taken, and into each 1 c.c. of undiluted serum is very carefully measured. To the one tube 9 c.c. of saline is added, to the other 4 c.c. of saline and 5 c.c. of the 5,000 million bacterial emulsion. The first, or control tube, will now contain a tenfold dilution of the serum only; the second, a similar dilution of the serum with the addition of an absorbing dose of 25,000 million bacteria. If it be desired to try a smaller dose in addition, some of the bacterial emulsion may be diluted down to 200 million per c.c., and 5 c.c. of this, with 4 c.c. of saline, added to 1 c.c. of serum in a third tube, thus giving an absorbing dose of 1,000 million per c.c. of serum.

The contents of the tubes having been well mixed they are left for 2 hours at room temperature, and then at 37° C. for an hour. They may then be centrifuged at once or after standing all night at room temperature.

The titre of each tube is now ascertained for any desired number of bacterial species or races, including of course the absorbing organism and the species from which the serum has been prepared. The most exact readings possible should be secured: Dreyer's technique lends itself particularly well to work of this kind. The results can be displayed best in graphic form, either as actual titres or as percentage reductions in titre for each strain tested.

It may be of service to give an example of this mode of working taken from actual practice. Flexner's dysentery bacillus presents a number of different serological races which may be distinguished by the letters *V*, *W*, *X*, *Y*, *Z*. These races overlap somewhat, group agglutination being often well marked. A certain unknown strain, which we will call *C*, is found to agglutinate with

a *V* serum, but also, though to a lesser degree, with a *W* serum. It is desired to ascertain for certain whether it belongs to the *V* or the *W* group. This can be done by absorbing a *V* serum and a *W* serum with large doses of the unknown strain and seeing which of them is exhausted of all its agglutinins. The results are shown in the following tables and diagrams. In the first column of each table is shown the titre of the serum in question before absorption for five different dysentery strains, including the homologous strain and the unknown strain *C*. In the second and third columns are shown the corresponding titres of the serum after absorption by small and large doses of *C* respectively. The titres have been calculated from the observed readings by Dreyer's reduction table (see p. 126):

A. Absorption of a *V* Serum by an Unknown Strain *C*.

	Unabsorbed serum.	After absorption with 1,250 million bacilli per c.c. serum.	After absorption with 58,000 million bacilli per c.c. serum.
Titre against a *V* strain . . .	1 in 1,132	1 in 720	1 in 31
,, ,, homologous *V* strain	1 in 1,132	1 in 824	1 in 33
,, ,, unknown strain *C* .	1 in 2,120	1 in 1,016	1 in 59
,, ,, a *Z* strain . . .	1 in 125	1 in 50	0
,, ,, a *W* strain . . .	1 in 233	1 in 125	0

B. Absorption of a *W* Serum by an Unknown Strain *C*.

	Unabsorbed serum.	After absorption with 1,000 million bacilli per c.c. serum.	After absorption with 32,000 million bacilli per c.c. serum.
Titre against a *V* strain . . .	1 in 59	0	0
,, ,, unknown strain *C* .	1 in 112	1 in 17	0
,, ,, a *Z* strain . . .	1 in 112	1 in 104	1 in 59
,, ,, a *W* strain . . .	1 in 896	1 in 530	1 in 530
,, ,, homologous *W* strain	1 in 498	1 in 360	1 in 360

The sera have been tested against a number of different strains in order to see whether all their agglutinating powers or only some have been virtually extinguished. In the case of the *V* serum it is seen that all the agglutinating power is gone after the heavy absorption, or reduced to something very small. In the case of the *W* serum the power of agglutinating a *V* strain and the unknown strain *C* is lost, but the reduction in titre for a *Z* strain and two *W* strains has only been moderate in degree. By reducing the figures to percentages of those yielded by the unabsorbed serum in each case, and plotting them as diagrams, this becomes still more clear. The unknown bacillus has absorbed both primary and secondary agglutinins from the *V* serum: in the *W* serum it has extinguished only the agglutinins for itself and the *V* strain. It has thus behaved as a *V* and not as a *W* strain, and therefore, as might have been anticipated from the way in which it originally agglutinated, it is proved to belong to the *V* group. The graphs (Figs. 14 and 15) incidentally illustrate the additional emphasis given to the results by employing a small as well as a large absorbing dose of bacteria.

(3) Multiple Simultaneous Absorption.

There are occasions when it may be of advantage to absorb a serum simultaneously with more than one bacterial strain. If,

for example, an investigation is being pursued into the antigenic structure of the different serological races of a given bacterial species, it may have been determined, by agglutination and absorption tests, that three antigenic components are present, each predominating in

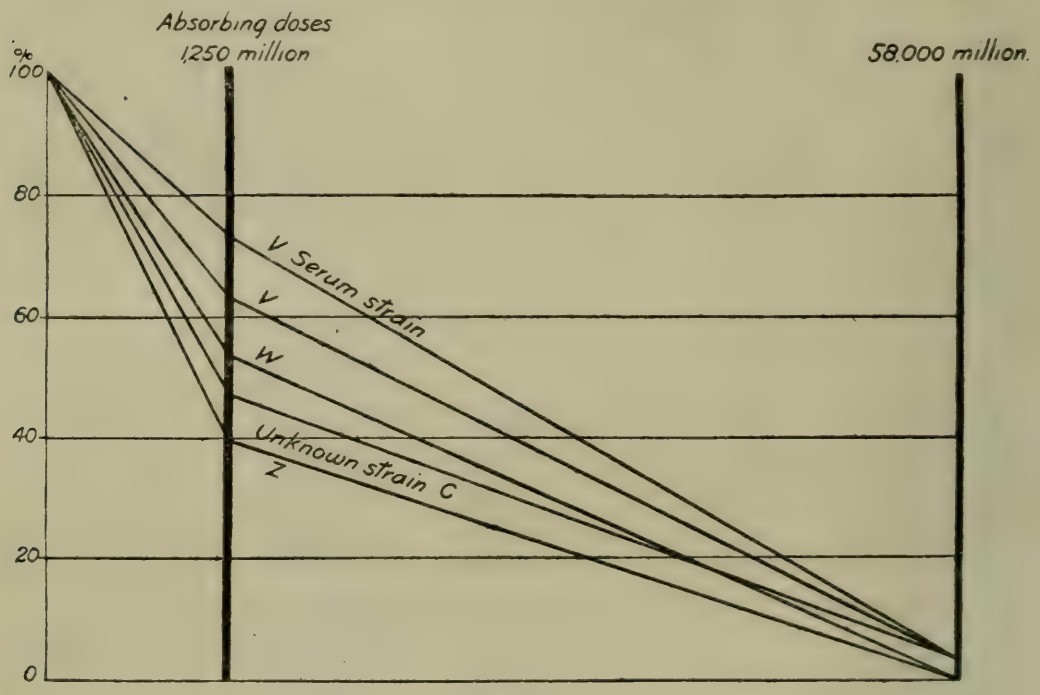


FIG. 14.

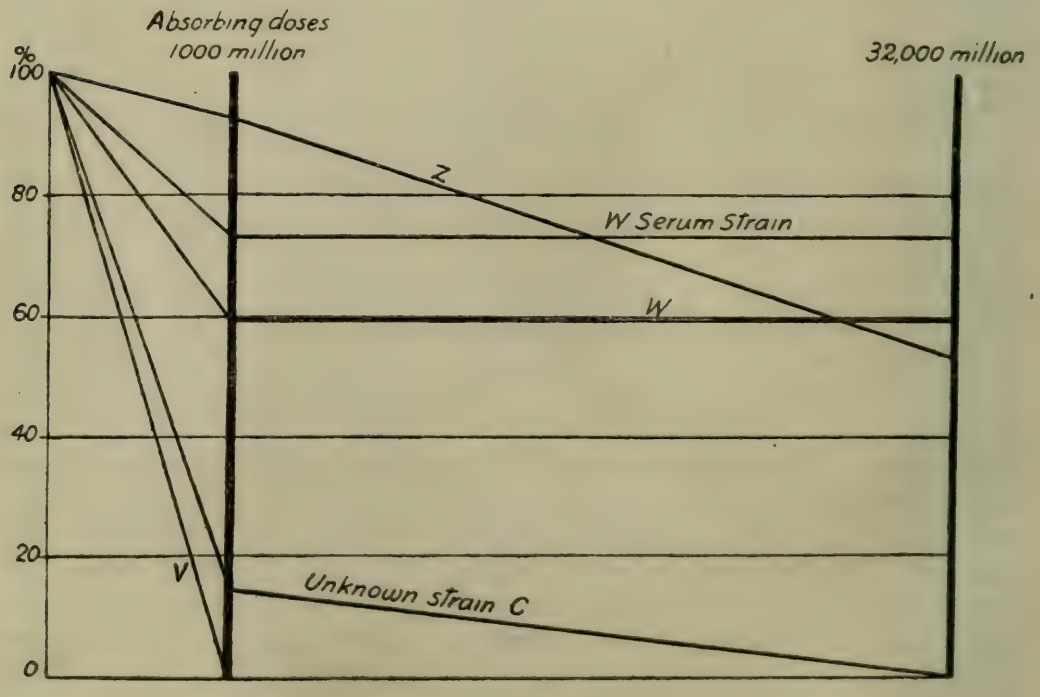


FIG. 15.

particular races. A fourth race may be encountered which appears more or less heterologous with the three races already established. The question, whether or not a fourth antigenic component is present in this race, is most readily answered by saturating a serum

prepared from it with typical examples of the three other races simultaneously. If its agglutinins are completely absorbed the presumption is against a fourth component: a considerable amount of residual agglutinin suggests the presence of a fourth component.

(4) Reciprocal Absorption.

The one-sided absorption of a serum by another race of bacteria may not always be sufficient to establish absolute identity between the two. For example, race *A* may contain three antigenic components (*a*, *b*, *c*), and race *B* may contain four (*a*, *b*, *c*, *d*). Serum *A* will be completely exhausted by saturation with race *B*, but serum *B* will be only partially exhausted by saturation with race *A*. Kruse pointed this out in his serological work on the dysentery bacilli, and there can be no doubt of its truth in certain instances. In order, therefore, to establish the identity of two strains beyond possible doubt, it may be needful to carry out reciprocal absorption. If strain *A* can completely exhaust serum *B*, and race *B* can do the same for serum *A*, there can be no doubt as to their complete identity.

IV. The Preparation of Agglutinating Sera.

Specific agglutinating sera for many of the common pathogenic organisms are issued by various institutions or may be purchased on the market. While these are for the most part reliable, the laboratory worker is often unacquainted with the precise character of the bacterial strains which have been used in their preparation. In any case, as soon as he commences any research involving the serological characters of any group of organisms, it becomes almost essential for him to prepare his own sera.

Of the various animals available for the purpose, the rabbit is not only the most convenient but probably the best. It readily yields a serum of good titre, and experience has shown that such rabbit sera are usually more sharply specific in their properties than sera from the horse or goat, while the yield of serum from smaller mammals is insufficient. The immunization of horses is a specialty lying outside ordinary laboratory work, and the rabbit alone will be considered here. It must be borne in mind, however, that the sharply specific antibodies formed by the rabbit, invaluable as they are for diagnostic purposes, may not necessarily have their precise counterpart in other animals or in man, though there is little to show that any essential difference exists between the rabbit and man as regards the nature of the immunizing response.

In choosing rabbits for immunization many prefer young animals. Gordon and Hine, in preparing agglutinating sera for the meningococcus, found that they got the best results from animals about one kilogramme in weight. Perfectly good antityphoid and antidysenteric sera can, however, be obtained from large adult rabbits of two kilos or more; aged animals should be avoided. The animals should be weighed periodically; it is best to use an animal which is gaining weight. Its condition during immunization may thus be gauged. If the intravenous route is to be employed

it will be found advantageous to select animals with large ears, as intravenous injections, or withdrawal of small quantities of blood, are much easier in such. When rabbits are purchased for immunization it is well to make sure that they have not previously been used for this purpose, as dealers sometimes offer animals which have been discarded from other laboratories. Before commencing immunization a small quantity of the rabbit's blood should always be tested for 'natural' agglutinins against the bacterium which is to be used and allied species. By taking this precaution one will know whether any heterologous 'coagglutinins' found later were due to the immunization or were already present.

(1) The Route of Immunization.

There is a choice of three routes, the subcutaneous, the intraperitoneal, and the intravenous. Administration by the alimentary canal is uncertain in its results and usually ineffectual. The subcutaneous route, though effectual, is slower than the others. When living bacteria are employed, this route, curiously enough, seems more liable to lead to a fatal result than the intraperitoneal or intravenous, while if killed cultures are injected there is the danger of abscess formation. The intraperitoneal route is safer in these respects. Most workers, however, prefer the intravenous method to either of the others, for a higher agglutinin titre is usually obtained in a shorter time and with a smaller immunizing dose. The intravenous route is therefore that recommended by the Committee for routine purposes, though excellent results can be obtained by subcutaneous injections. The former has one possible drawback, namely, the risk of anaphylactic shock when too long an interval has been allowed to elapse between successive doses: if, however, the technique about to be described is followed, the risk is almost non-existent, though in the preparation of haemolytic sera it often gives rise to serious trouble.

If, for any reason, the subcutaneous route is preferred, the injection is best made over the pectoral muscles, on the inner side of the thigh or in the groin, and the needle should be sharp: the skin of a rabbit's back is so tough and mobile that a blunt needle may easily be broken off.

(2) The Form of Antigen.

Living bacteria are often well tolerated when introduced into the circulation, and their employment offers a guarantee that the bacterial protein is unaltered. There is, however, always some risk of death from septicaemia, and, as equally good results appear to follow the use of killed cultures, the latter are usually preferred. If killed by heating, great care must be exercised to avoid overheating; the temperature with some species, e.g. *B. typhosus*, should not have exceeded 57° C., or there may be loss of antigenic properties. In the case of the meningococcus, Gordon and Hine got good results with emulsions heated to 60° C. Many prefer to use cultures killed in other ways. A broth culture, with the addition of 0·5 per cent. carbolic acid, and placed at 37° C., is

usually dead in 24 hours, or, as Dreyer recommends, 0·1 per cent. formalin may be added and the culture put in the ice chamber when it is found dead in four or five days. Cultures thus treated are excellently adapted for immunization purposes.

(3) The Immunizing Dose.

In order to obtain sera of good titre a proper spacing of the doses is of more importance than their size. Small doses, properly administered, are more effectual than large ones unwisely spaced. A good method consists in administering a second and third dose just before, or at least as soon as, the titre of the serum resulting from the preceding dose has begun to decline. A single intravenous dose of killed bacteria is found, when its effects are watched from day to day, to produce a rise in the agglutinating titre of the serum which reaches its acme about the sixth day, after which it falls rapidly and steeply. So constant is this curve that one may take it almost as a rule of thumb to administer the second dose 5 or 6 days after the first. An alternative, and sometimes more effective, method, consists in administering small equal doses at intervals of two days. The number of doses will be governed more or less by the titre required and by the response of the individual rabbit, as animals are well known to differ greatly in the degree to which they respond. There is little advantage in sera of enormously high titre: for practical purposes a titre of 1 in 2,000 or 1 in 4,000 is ample, and this can usually be secured by two immunizing doses of quite moderate size. Even a single dose of typhoid or dysentery bacilli will often yield a titre of 1 in 1,000 by the 5th or 6th day, and this may be quite sufficient for all purposes.

The size of the doses administered is often unnecessarily large. In most cases a few hundred million bacteria produce an excellent immunizing response, and such a dose is far less likely than a large one to cause illness or death in the animal. A dose which renders the rabbit very ill is less likely to yield a good immunizing response than a smaller one. In the case of typhoid and paratyphoid bacilli and of Flexner's dysentery bacillus half a cubic centimetre of a killed 24-hour broth culture, followed on the 5th or 6th day by the same or a slightly larger dose, will usually yield a serum of adequate titre by the 10th or 11th day. Murray uses doses of 500 million and 1,000 million dysentery bacilli, and states that he rarely failed to secure a titre of 1 in 8,000 by the 10th day. Greater difficulties have to be encountered when the immunizing bacterium is one of high toxicity. This is a universal experience with Shiga's dysentery bacillus, of which the dosage must be small (20 to 50 millions killed bacilli) and the effect carefully watched. The illness induced is often severe, and the sick rabbit may require as careful nursing and diet as a human patient. Indeed, care in this respect often plays a considerable part in successful immunization against other bacteria, and it is well that the bacteriologist should personally overlook the comfort of his animals. In the case of Shiga's bacillus it has been found that heating the bacteria to

80° C. for an hour materially reduces their toxicity while still leaving sufficient immunizing power.

(4) The Technique of Bleeding.

In watching the progress of immunization it is necessary to remove small quantities of blood from time to time to ascertain the titre of the serum. This is readily done from the marginal vein of the ear. The vasomotor system of a rabbit's ear is a very active one, and in order to secure a due yield of blood it is necessary that the vessels should be well dilated. The rabbit should be enveloped in a towel to prevent sudden movements, its head alone being free. Vascular dilatation may be promoted by friction of the ear or by shaving its margin or by holding it near the bulb of an electric light. Better still is the application of ether or xylol by means of a swab, although if the latter is used the ear must be subsequently washed. As soon as the marginal vein is seen to be well dilated, and this usually requires a little patience, the necessary amount of blood can be withdrawn by syringe, or the vein may be pricked with a thick, sharp, short-bevelled hypodermic needle and the blood allowed to drip into a small test tube. The vein should be compressed at the base of the ear by an assistant or by means of a light clip.

As soon as examination of the serum shows that its agglutinating titre has reached the desired point the animal should be bled out as soon as possible, otherwise the titre will fall rapidly. If the bleeding is to be done from the carotid artery the following apparatus should be in readiness: scissors, scalpel, and forceps; two or three pairs of small artery clips and a blunt probe or 'seeker'; test tubes and a small beaker with cotton wool at the bottom for the anaesthetic. Ether is a good anaesthetic and should be administered freely so as to get the animal quickly under. As soon as anaesthesia is complete, the animal is laid on the back and the hair on the neck removed with scissors. A long incision is then made in the middle line from the chin to the sternum, best by means of scissors. The superficial tissues are pulled aside and a little dissection on one side of the trachea reveals the carotid artery, which should be cleared from its surroundings for an inch and a half. This can be done with a blunt instrument: the less the scalpel is used the better, as haemorrhage is thus avoided. Two clips are put close together on the vessel at the upper end and a third at the lower end of the incision. The artery is divided between the two upper clips, leaving them on the cut ends. The lower of these two, now closing the upper end of the artery, is brought out, so as to hang down with the artery into the mouth of a test tube, and a small nick is made in the vessel close to the clip. The third or proximal clip is now released, when the blood streams into the test tube: a second tube should be in readiness when the first is full. As the stream lessens pressure should be kept up on the abdomen to empty the splanchnic vessels, and the animal may be held up by the hind legs. An assistant is of course required to look after the anaesthetic and help generally. The

yield of serum from a rabbit of moderate size should be not far short of 40 c.c. The yield may be materially increased by interrupting the flow for periods of 2 or 3 minutes every 15 or 20 seconds.

The blood should be allowed to clot with as little disturbance as possible. When firmly clotted it should be gently detached from the wall of the test tube and left for 24 hours for the clot to shrink. The shrinkage is hastened by placing the tube for an hour in the incubator at 37° C., after which it should be kept over night at room temperature; an improved method is described below (see p. 149). The serum is pipetted off, and if there is any accidental admixture of blood corpuscles it is centrifuged. Finally, a preservative is added, as a rule 0·5 per cent. phenol; to every 9 c.c. of serum 1 c.c. of 1 in 20 carbolic is required. This causes a slight precipitate which soon settles down. It is better to bottle the serum in several small rather than in one large bottle so as to minimize the risk of contamination.

Serum should be stored in the dark at low temperature, preferably in the ice chamber. Its initial titre is apt to undergo a marked fall during the first few weeks, after which it remains stable for many months or even years.

We owe to Dreyer and Gardner a method of obtaining large quantities of blood from rabbits without killing them. The animal, having been suitably anaesthetized, is tightly rolled up in a large cloth in such a way that no movement of the body or limbs is possible. Particular attention should be paid to the upper part of the thighs and pelvis, round which the cloth is wound tight and firmly pinned. The forelegs should be held down along the chest and the cloth wound so as to prevent their protrusion. No attempt at a full bleeding should be made without taking these preliminary measures.

The fur of a portion of an ear over the marginal vein is clipped or shaved. In cold weather, or if the ear is cold, it is warmed by rubbing and washing with hot water and soap. The dilatation of the veins is of considerable importance for a successful bleeding. The clipped area of the ear is further sterilized with volatile antiseptics, such as alcohol and ether. Sterile vaseline is smeared with a sterile instrument over the prepared area and the margin of the ear. If the marginal vein is now no longer dilated, a drop or two of xylol may be dropped upon the tip of the ear, but it must not reach the prepared area, as it is a haemolytic agent.

For puncturing the vein a sterile, round, hollow needle of as large size as possible is used. It should be just able to travel along the vein, and its point should be short, not tapering too much. It is best to direct the puncturing needle away from the heart and towards the periphery. The needle should be pushed right into the vein, avoiding damage to the walls, and should then be withdrawn with a slight upward pressure, calculated to open wide the puncture hole.

If the vein be fully dilated and the puncture a good one, blood will flow either continuously or at a very rapid drop-rate, and 40 c.c. will be obtained in 5–10 minutes. Frequently, however, a contraction of the veins occurs, either as a result of the puncture,

or at a later time, and here some patience is needed. In such a case it is neither desirable nor efficacious to make further punctures, but dilatation of the veins should be induced by a gentle scratching of the ear, or, if this fails, by applying a drop or two of xylol to the tip. The flow may often be accelerated by passing the needle again through the puncture hole, to re-open it, and to clear away any tiny clot that may have formed at its edges.

By such measures it is always possible to obtain 30–50 c.c. from a rabbit of reasonable size, though it may take half an hour if much difficulty is experienced.

The blood soon attains its original volume, and so the whole process may be repeated on the following day. Moreover, a third bleeding within a few days from the second may be done without endangering the life of the animal. Plenty of water must be provided for the animal to drink during these times, in order that it may replenish its body fluids. In the case of agglutinating sera there will be some loss of titre in the later bleedings, but this does not appear to exceed 50 per cent. at most, and is probably much less as a rule. A single rabbit may in this manner be made to yield more than 100 c.c. of blood within three or four days, and it will be alive and, after an interval for recuperation, perfectly fit to be re-inoculated and bled for an indefinite number of times. An enormous economy of cost and of animal life is insured by the use of this method.

The sera thus obtained are efficiently preserved by the addition of 0·3 per cent. of phenol. It is preferable to add already diluted phenol, to minimize the precipitation of serum proteins. The quantity of a phenol-solution to be added to any given volume of serum may readily be calculated by the use of the following formula, which is applicable to mixtures generally,

$$Q = \frac{Vp}{P-p},$$

where Q is the quantity of phenol solution to be added, V is the volume of serum to which it is to be added, p is the percentage concentration of antiseptic desired, P is the percentage strength of the phenol solution used.

In this particular process, if we adopt 0·3 per cent. always for p, and 5·0 per cent. for P, then $\frac{p}{P-p}$ is a constant and equals ·064 approximately. Thus $Q = \cdot 064\ V$.

A method of obtaining a certain and good yield of serum from coagulated blood with a minimum of manipulation and of consequent haemolysis has been described by A. D. Gardner.[1]

A constant source of trouble in the making of immune sera is the failure of coagulated blood to separate and contract from the walls of the containing vessel. Sometimes contraction and separation do not occur at all, sometimes they occur partially, and in either case the result is delay in obtaining a proper yield of

[1] A. D. Gardner, *Lancet*, 1917 ii, 51.

serum and the necessity for manipulations which cause breakage of corpuscles, and a consequent and undesirable liberation of haemoglobin into the fluid.

The following procedure has proved successful in overcoming these difficulties, and in insuring an excellent and sure yield of serum free from haemoglobin.

Method.—The inside of the tubes or cylinders or other vessels which are to receive the blood are lined with the thinnest possible continuous coating of agar dissolved in physiological saline solution:

Agar. 1½ per cent. in 0·85 per cent. NaCl solution.
No alkali is added.

A sufficient quantity (e. g. 1·0 c.c. for an ordinary test tube) is poured into a number of tubes, which are then plugged and autoclaved. Shortly before use the agar is melted and allowed to run over the whole inner surface of the tube (which should be warmed). Excess of agar is poured away. Directly the agar has set into a very thin and perfectly continuous coating, the tube is ready for use. Coated tubes can be used even after the agar skin has dried completely, i. e. after days or weeks.

Mechanism.—The clot adheres to the agar skin. Serum filters through and separates the skin from the glass. The skin, when it remains perfect, keeps corpuscles in and lets serum out. The clot remains suspended from the top and draws up from the bottom of the tube. Failure to separate is exceedingly rare and due to imperfect coating with agar.

Yield.—The average yield of serum from agar tubes is equal to the maximum yield from plain glass when separation of the clot from the latter is complete.

V. Acid Agglutination.

The fact that bacterial suspensions could be agglutinated by the action of acids, quite independently of the presence of the agglutinating serum, has been known for many years. Dreyer and Jex-Blake,[1] who worked with *B. coli* and *B. typhosus*, employing agar suspensions and broth cultures, pointed out the marked inhibition zones which may attend the phenomenon. A given emulsion may be agglutinated by strong and by very weak concentrations of acid, and not by intermediate strengths; the width of the inhibition zone varies with the density of the bacterial emulsion, becoming less or even disappearing as the emulsion increases in density. These writers also noted that emulsions which had been dialysed free from salts, though no longer agglutinable by immune sera, were still flocculated by acids, and further that the presence of normal horse serum modified the acid agglutination in various ways.

There is no reason for doubting that the process of acid agglutination is identical with the second phase of agglutination by an immune serum, namely, the flocculation of the sensitized

[1] *J. Path. and Bacteriol.*, 1906, 11, 1.

bacteria by an electrolyte, but it occurs without any preliminary sensitization. It appears to depend upon H-ion concentration, and the view that the positive charges borne by the hydrogen-ions neutralize the negative charges borne by the suspended bacteria and thus abolish mutual repulsion may be taken as a good working hypothesis, though there is much about the matter not yet satisfactorily explained.

No serious attempt was made to utilize acid agglutination in practical diagnosis until 1915, when Michaelis[1] pointed out that different bacteria reacted differently in a series of acid solutions presenting an ascending scale of H-ion concentrations, care being taken as far as possible to exclude other electrolytes. He recommended the following six solutions:

	Normal caustic soda.	Normal acetic acid.	
Sol. 1	5 c.c.	7·5 c.c.	Each made up with distilled water to 100 c.c.
2	5 c.c.	10 c.c.	
3	5 c.c.	15 c.c.	
4	5 c.c.	25 c.c.	
5	5 c.c.	45 c.c.	
6	5 c.c.	85 c.c.	

The solutions were to be used in the following manner: A well-grown 24-hours agar culture of the bacterium to be tested is emulsified in 20 c.c. of distilled water, and 3 c.c. of the emulsion placed in each of six test tubes. One c.c. of each of the six solutions is added to the several tubes and well mixed. The tubes are now incubated for two hours at 37° C. and then inspected: in the case of the typhoid bacillus the maximum agglutination is asserted to be in tube 3, rarely in 2 or 4, whereas with paratyphoid bacilli it is in tube 5 or 6. Dysentery bacilli were found to be unagglutinated in any tube of the series, and the same was usually true of *B. coli* though occasional strains of *B. coli* behave like *B. typhosus*. In a later publication,[2] Michaelis states that if a trace of serum be added to the mixture *B. coli* is always agglutinated, but that dysentery bacilli are still unaffected.

The value and limitations of this test are still under observation and discussion. Some writers have found Michaelis's statements substantially correct: others are unable to confirm them. It would appear that laboratory strains long under cultivation are apt to give less uniform results than strains recently isolated.

Hirsch[3] has repeated Michaelis' observations with certain modifications in the technique, the chief being a washing of the bacilli, which were then centrifuged out and again suspended in distilled water. In this way traces of electrolytes from the agar were practically eliminated. The quantity of serum added to each tube was 0·3 c.c. of a 1 per cent. dilution: the serum had previously been heated for 4 hours to 60° C. on 3 successive days. Under these circumstances *B. coli* was always agglutinated, especially in tubes 5 and 6. Dysentery strains to the number of 143 were similarly tested: 70 per cent. were not agglutinated at all: the remaining 30

[1] *Deutsche med. Wchnschr.*, 1915, 41, 244.
[2] Ibid., 1917, 43, 1506.
[3] *Med. Klin.*, 1918, 14, 932.

per cent. gave weak agglutination, only faintly flocculär, and limited to tube 1 except in a single instance—a so-called 'Strong' strain which agglutinated up to tube 4. This weak agglutination was seen in 38 out of 92 Shiga strains, in 1 out of 38 *Y* strains, in 2 out of 9 Flexner strains, and in 2 out of 4 Strong strains. The agglutinable strains, with few exceptions, were old laboratory cultures: freshly isolated dysentery cultures were almost all inagglutinable.

P. Eisenberg[1] employed a technique differing in two important respects from that of Michaelis. He extended the range of H-ion concentration, using 10 tubes, the last four of which were more highly acid than Michaelis' solutions, and he increased the time of incubation to 3–5 hours and finally to 15–16 hours at 37°. It is therefore not surprising that he only partially confirmed Michaelis' conclusions. Nevertheless his results confirm those of Michaelis in 86·4 per cent. out of 163 typhoid strains, in 77 per cent. of 9 paratyphoid *A* strains, in 73·9 per cent. of 54 paratyphoid *B* strains, and in 69·7 per cent. of 86 various dysentery strains (Shiga, Flexner and *Y*). If agglutination at higher H-ion concentrations, such as Michaelis did not employ, be disregarded, the correspondence with Michaelis' results is much greater: thus 94 per cent. of his dysentery results would have been correctly diagnosed by the strict Michaelis technique. Eisenberg concludes, probably with justice, that acid agglutination is not a perfectly constant specific criterion, and hence not a diagnostic test upon which complete reliance can be placed.

Andrewes,[2] using the original Michaelis technique, and adding to each small tube (2 c.c.) one drop of tenfold dilution of normal human serum, unheated, tested 9 strains of Shiga and 29 strains of Flexner and *Y* bacilli. No Shiga strain showed more than slight granularity with a lens, and only 2 of the Flexner strains showed very weak agglutination. On the other hand, 13 strains of the indol forming species allied to Shiga's bacillus (which he terms *B. ambiguus* and which is perhaps identical with Schmitz's 'dysentery bacillus') all gave strong acid agglutination, as did 11 dulcitol fermenting strains (*B. alkalescens*) and 11 lactose fermenting strains of the dysentery group (*B. dispar* group) together with 12 strains resembling Flexner, but inagglutinable with Flexner and *Y* sera. These acid-agglutinable forms, some of which may be concerned in the causation of dysentery, are precisely those most liable to be confounded with the bacilli of Shiga and Flexner.

Murray,[3] on the contrary, regards Michaelis' test as of no value in the diagnosis of dysentery bacilli. The chief reason for this opinion lies in the fact that he includes in this group a number of lactose fermenting forms, and the indol forming bacillus of Schmitz. When his data as regards the bacilli of Shiga and Flexner are examined they are not found very divergent from those of Hirsch and Andrewes.

A consideration of the facts so far ascertained with regard to Michaelis' application of acid agglutination to practical diagnosis

[1] *Centralbl. f. Bakt.*, 1919, Orig. **83**, p. 70.
[2] *Lancet*, Lond., 1918, i, 560.
[3] *J. Roy. Army Med. Corps*, Lond., 1918, **31**, 257 and 353.

suggests the following conclusions. The cultural and serological distinctions amongst the typhoid, paratyphoid, and colon bacilli are so manifest that the test of acid agglutination is in practice superfluous and is unlikely to come into general use, whatever its theoretical interest may be. Its real utility lies in the discrimination between Shiga's and Flexner's bacilli on the one hand and closely allied species which equally form no gas in glucose media. Here it offers a ready test, though probably not an absolute one, since all workers have noticed a few exceptions to the rules that Michaelis laid down. If attention be paid only to gross flocculation the test appears one of considerable value, and for the purpose named there seems little need to complicate it by the addition of a trace of serum, since no intelligent bacteriologist is likely to confound *B. coli* with a member of the dysentery group.

APPENDIX

NOTES ON THE HISTORY OF THE STANDARDIZATION OF AGGLUTINABLE BACTERIAL EMULSIONS.[1]

By A. D. Gardner, M.D., F.R.C.S.

The development of a true standard for the quantitative measurement of the agglutinating power of a serum, and the translation of the idea into practice were the fruits of Prof. G. Dreyer's researches in Denmark during the earliest years of the present century. Madsen and others had done valuable pioneer work in the direction of the accurate quantitative measurement of agglutinins, but it remained for Dreyer both to eliminate certain sources of errors in actual technique and, most important of all, to establish a basic standard of universal applicability whereby titrations of agglutinating sera could be made and expressed in figures (agglutinin units) having a fixed and definite though arbitrary significance. Hitherto the agglutination test had been an approximate measurement of one unknown quantity (the agglutinating power of the serum) by means of its degree of reaction with a second unknown quantity (the agglutinability of the bacteria used). This latter had been held to be sufficiently constant for the purpose of clinical tests, but it was already known by the Danish school that it was too variable to permit the employment of freshly made suspensions of living bacteria in any series of experiments where it was necessary to compare quantitatively one day's results with those of another.

An extensive series of experiments by Dreyer and Jex-Blake, published in 1905 and 1906, laid the foundation of more exact knowledge of certain properties of agglutinable emulsions of bacteria which enabled Dreyer in 1906 to introduce the use of bouillon emulsions of typhoid bacilli, &c., killed and fixed in the cold with minimal quantities of formaldehyde, such as he had already employed for some time both in routine and in experimental work.

The essential properties of these cultures are (1) sterility, (2) absence of any excess of antiseptic in the fluid, (3) sensitiveness substantially equal to that of living cultures, (4) permanency of this sensitiveness. These properties render the cultures suitable for standardization, i.e. 'They render possible the production of a standardized culture of a given constant agglutinability'.

At the time Dreyer thought it advisable to make the cultures issued from the Danish State Serum Institute all of the same degree of agglutinability, in order to avoid the introduction of a correcting factor for each batch of culture. This was done by a process of dilution of the culture with saline (to increase sensitiveness) or with the filtrate from similar cultures (to decrease sensitiveness) in successive steps controlled by comparative tests against a stock

[1] This was written at the request of the Committee.

standard culture. The cultures thus prepared were not of uniform opacity; for it is a matter of little importance to maintain uniformity in this respect provided that constant agglutinability is assured.

It was, however, only in these cultures for public issue that opacity-standardization was, for greater convenience, dispensed with. Repeated references to careful opacity tests are made in the paper by Dreyer and Jex-Blake already quoted. Moreover the whole technique employed for years by Dreyer in experimental work on agglutination is described by Schroeder in 1909, and it is there seen that the fullest attention was paid both to measurements of opacity and to estimations of sensitiveness of all emulsions used. The dates of the experiments in this paper go back to 1905, at which time, therefore, Dreyer's technique was already complete. Since then no significant alterations have been found necessary.

The use of standardized cultures had the logical consequence of the introduction of a standard agglutinin unit. For if a serum be tested with two emulsions of different sensitiveness, the apparent titre of the serum in each case will be in direct proportion to the sensitiveness of the emulsion used. For example, a typhoid serum is tested with two emulsions *A* and *B* of the typhoid bacillus. After a given period of incubation at a given temperature the same degree of agglutination (strong flocculation without sediment) is found in the case of emulsion *A* at 1/1000, and in the case of emulsion *B* at 1/1800. Clearly *B* is more sensitive than *A*, and in the proportion of 1·8 to 1·0. If these two tests had been performed quite independently by different observers (ignorant of differences of agglutinability) the one observer would have called the titre of the serum 1/1000, the other would have given it as 1/1800. Thus there would be a complete lack of agreement in the measurements of the same serum by two workers. But it is a simple matter to introduce order into such a chaos by the use of a reduction factor expressing the precise degree of agglutinability of each emulsion as compared with that of an original, arbitrarily chosen, whose agglutinability is taken *as standard*. In the case quoted let us suppose that emulsion *A* is the original standard culture and *B* some other emulsion. The agglutinability of *A* may be called 1·0, in which case the agglutinability of *B* is 1·8. Now it is at once evident that if we divide the denominators of the respective dilution fractions by the figures expressing the agglutinabilities of the two emulsions, we arrive at the same figures in each case, for $1{,}000 \div 1{\cdot}0 = 1{,}000$ and $1{,}800 \div 1{\cdot}8 = 1{,}000$. Here we have a method by which we can arrive at the same 'titre' for a serum by the use of emulsions of different but known sensitiveness. The titre can no longer be expressed in dilutions, but must be stated in standard agglutinin units.

In the case given the serum can be said to contain 1,000 standard agglutinin units 'per c.c.'. The last words 'per c.c.' need explanation. It has been assumed that the tests were done according to Dreyer's technique, which is fully described in Schroeder's paper, and later by Dreyer and Douglas and A. D. Gardner. This technique is so arranged that the relative volumes of serum dilution and of bacterial emulsion are present in each tube in the proportion of 1·0 to 1·5, it matters not what method of measurement is employed nor what magnitude of volume is chosen. Thus if 1·0 volume of diluted serum is found to have a given action on 1·5 volumes of emulsion, we can state that 1·0 c.c. of the diluted serum will have that same action on 1·5 c.c. of the emulsion. This circumstance makes it possible to state the number of units per c.c. of serum, although in the actual test the measurements have been made by the drop method or by any other method of measurement that dispenses with the metric unit.

It is from the arrangement of the technique also that the definition of the standard agglutinin unit arises. This is defined as:

'That amount of serum which, when made up to 1·0 c.c. volume with normal saline solution causes standard agglutination on being mixed with 1·5 c.c. of a particular standard agglutinable culture and maintained at 50°–55° C. for 2 hours (dysentery cultures 4½ hours) in a water-bath, followed by 15 to 20 minutes at room temperature.'

It is to be noted that 'standard agglutination' is defined as marked agglutination, without sedimentation, and there will be found below the description

of a means whereby this precise degree of agglutination may be accurately and permanently fixed.

From the definition of the standard unit arises the fact that the reduction factor (K) for the originally chosen standard culture must theoretically be 2·5 and not 1·0. For if 1 c.c. of a serum (undiluted) gives standard agglutination with 1·5 c.c. of the culture, that serum contains *ex hypothesi* 1 standard agglutinin unit. But the serum has been mixed with 1·5 c.c. of culture, and is therefore acting in a dilution of 1/2·5. Now we have seen that, to obtain the number of agglutinin units in the serum, we have to divide the denominator of the dilution-fraction by the figure (K) representing the agglutinability of the culture used. In the case before us the sum is:

$$\frac{2{\cdot}5}{K} = 1 \text{ or } K = 2{\cdot}5.$$

When introducing above the subject of differences in sensitiveness of cultures we spoke, for the sake of simplicity, of the original standard culture A having the K of agglutinability 1·0. But we now see that, from the definition of the standard unit, it must be theoretically 2·5. Culture B, then, which was 1·8 times more sensitive than A, would have the K of 4·5.

For scientific work the absolute magnitudes given to the K's of successive cultures are a matter of no importance, provided that their ratios represent the true relative agglutinabilities of the cultures. But for the purpose of practical diagnostic tests it may be convenient to increase or decrease the absolute magnitude of all the K's for any particular species of bacillus, keeping, of course, their ratio unaltered. For instance, in the standardization of dysentery cultures an original K of 2·0 was fixed by Prof. Dreyer for *B. dysenteriae*, Shiga, and a K of 3·0 for *B. dysenteriae*, Flexner. The reason for this procedure was that normal human sera were found to agglutinate Flexner's bacillus more frequently and to a higher degree than they agglutinate the bacilli of the typhoid group. *B. dysenteriae*, Shiga, on the contrary, is practically never clumped by such sera even at a very low dilution.

With the K's fixed as above, a serum which agglutinates *B. Shiga* at a dilution of 1/20 is made to contain 10 standard units, whereas a 1/20 reaction to *B. Flexner* gives only 6·7 units; and it is not until an agglutination at a dilution of 1/30 is reached that a serum is said to contain 10 units for *B. Flexner*.

Thus, by arbitrarily increasing the value of the original K for a species of bacterium we can reduce the content in units of normal sera down to any limiting figure we wish. This enables us to introduce uniformity in the records of reactions of normal sera with different species of bacterium, e.g. to adjust the K of all cultures so that no normal serum contains so much as 10 standard units.

In thus arbitrarily adjusting the K, the 'scientific fiction' is adopted that there was an original standard culture of a K of 2·5, which thus satisfied the requirements of the definition of standard unit, and that the actual K of the first real culture was obtained from the fictitious standard culture by the usual process of standardization.

The standard agglutinin unit was employed in all the work of Prof. Dreyer and his co-workers and pupils from 1905 onwards whenever its employment was desirable. Schroeder's paper, as has been said, takes the use of the full technique back to that year, and subsequent publications by Dreyer and E. W. Ainley Walker, Dreyer and J. S. C. Douglas, &c., uniformly adopt the expression of the serum titre in standard units.

It may be mentioned that in Dreyer's paper on 'Widal's reaction with sterilized cultures', 1906, there is no direct mention of the unit, because the paper was intended for the general medical public, and the technique had been so arranged (by the preparation of cultures always with the same K, as mentioned above) as to dispense with the use of the unit. For when all cultures have the same K, the dilution figure itself is equivalent to a statement in units. Again, in a later paper by Dreyer, Walker, and Gibson all results are given in dilutions and no mention is made of units, although reference is made to the use of standard cultures. This paper, as the previous one, was directed to an immediate practical end, and it was considered desirable to

avoid confusing the reader by the use of the agglutin unit, with which he might be unfamiliar.

When the Medical Research Committee in 1915, at Prof. Dreyer's instigation, took in hand the manufacture of standard agglutinable cultures on a large scale for the Army and Navy, it was decided to issue each successive batch of culture (Typhoid and Paratyphoid) with its respective *K* of agglutinability on the label of the bottle. The cultures then could be made of constant opacity, and all that the user had to do was to make the simple calculation of agglutinin units for each test. The preparation of cultures of constant agglutinability on such a scale was considered impracticable.

At first only cultures of *B. typhosus*, *B. paratyphosus A* and *B* were issued. But in 1916, as a result of a research by A. D. Gardner, carried out at Prof. Dreyer's instigation and by means of his methods, the standardization was applied to a number of varieties of dysentery bacilli.

Incidentally, *B. enteritidis* (Gärtner) was included in the committee's list and several other bacilli, such as *B. Aertrycke* and *B. coli*, *Vib. cholerae*, were available for standardization should the occasion arise.

There is no limit to the applicability of the principle of standardization, for it can be brought into use wherever a micro-organism is susceptible of agglutination by a specific serum, when once the technical difficulties appertaining to the growth of the particular organism are overcome.

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8. Gardner, A. D., *J. Hyg.*, 1918, 17, 471.



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Fig. 1. *E*
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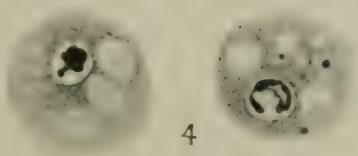
Fig. 2. *E*

Fig. 3. *E*
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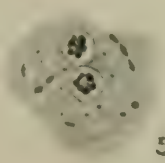
Fig. 4. *E*

Fig. 5. *D*

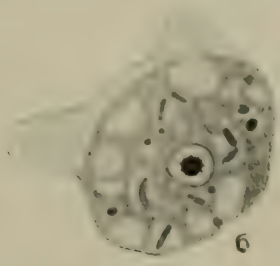
Fig. 6. *I*



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6

PLATE I

The amoebae of the human intestine. Drawn from fixed and stained specimens. × 2,000. From Dobell, 'The Amoebae living in man', 1919.

Fig. 1. *Entamoeba histolytica*; large active adult form, containing three partly digested red corpuscles.

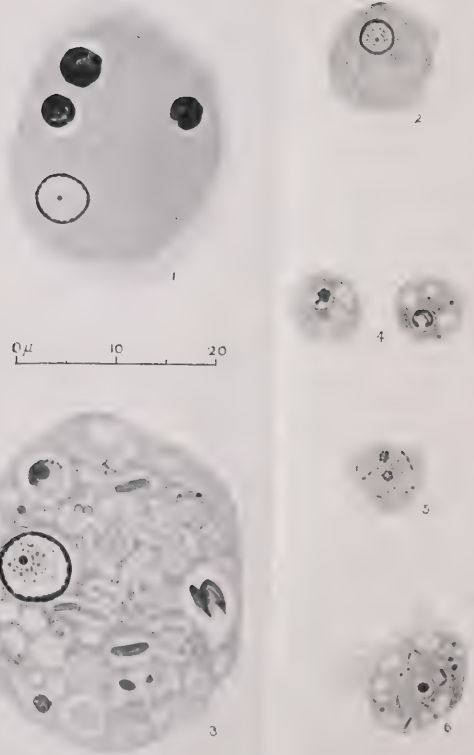
Fig. 2. *E. coli cloni*; small precystic form.

Fig. 3. *Entamoeba* [illegible]; large active adult form, containing numerous bacteria and other ingested food bodies.

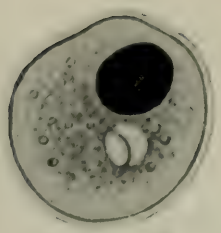
Fig. 4. *Endamoeba minuta*; two active individuals.

Fig. 5. *Entamoeba tetragena*; typical binucleate specimens.

Fig. 6. *Endamoeba coli blat*; active adult individual.



(Dobell del.)



9

Cysts of Amoebae, &c., occu

Fig. 7. Cyst of *E. histolytica* diameter of about 12 μ. In io shows the four nuclei, a chroma ill-defined outline).



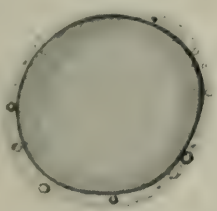
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Fig. 8. Cyst of *E. histolytica* size of about 7·5 μ. Fixed witl Four nuclei, two chromatoid bod are shown.

Fig. 9. Mature cyst of *I. bi* iodine solution. Note the dee and the single small signet-ring

Fig. 10. Mature cyst of *E.* contents, in which the nuclei ar

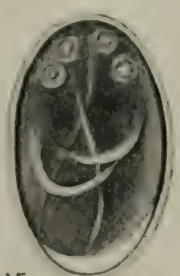
Fig. 11. A similar cyst of with haemalum. Note the fou No chromatoid bodies are presen



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Fig. 12. Mature, 8-nucleat specimen, showing neither chro

Fig. 13. Cyst of the flagel easily distinguishable from the *E. nana* (Figs. 10 and 11), by its are: (i) a single nucleus, usually the remains of buccal apparatus



15

Fig. 14. A living specime common in human stools. The surrounding an enormous more

Fig. 15. Cyst of *Giardia* (= of this flagellate usually contai pole; and in addition rod-like a parts of the free form (axostyles

PLATE II

Cysts of Amœbidæ, &c., occurring in human faeces. × 2,000.

Fig. 7. Cyst of *E. histolytica*; belonging to a race forming cysts with a mean diameter of about 12 μ. In iodine solution. The cyst is fully developed, and shows four nuclei, a chromatoid body, and a glycogen vacuole (dark area with ill-defined outline).

Fig. 8. Cyst of *E. histolytica*; belonging to a race with cysts having an average size of about 7·5 μ. Fixed with sublimate-alcohol and stained with haemalum. Four nuclei, two chromatoid bodies, and a vacuole (originally containing glycogen) are shown.

Fig. 9. Mature cyst of *I. bütschlii* ('I. cyst' or 'Iodine cyst' of Wenyon). In iodine solution. Note the deeply stained glycogen mass, with a sharp contour, and the single small signet ring nucleus.

Fig. 10. Mature cyst of *E. nana*, in iodine solution. Note the finely granular condition in which the nuclei are almost invisible.

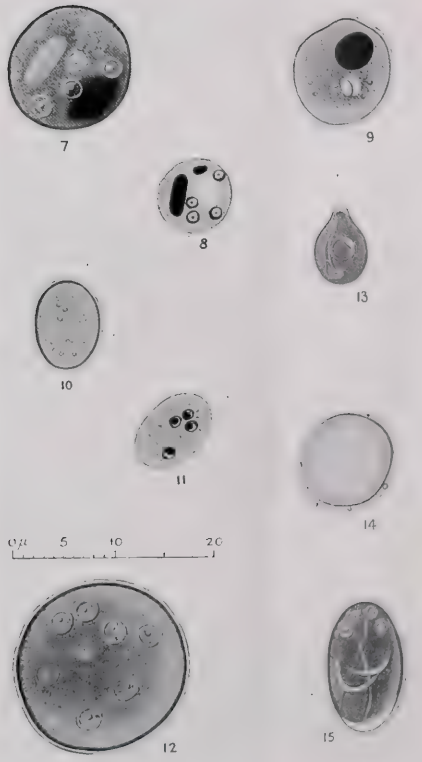
Fig. 11. A similar cyst of *E. nana*, as it appears after fixation and staining with haemalum. Note the four small nuclei, with large eccentric karyosomes. No chromatoid bodies are present (cf. Fig. 8) in the cysts of this species.

Fig. 12. Mature, 8-nucleate cyst of *E. coli*, in iodine solution. A typical specimen, showing neither chromatoid bodies nor glycogen (cf. Fig. 7).

Fig. 13. Cyst of the flagellate *Chilomastix mesnili*, in iodine solution. This is easily distinguishable from the small cysts of *E. histolytica* (Fig. 8), and those of *E. nana* (Figs. 10 and 11), by its shape—like a lemon—and its inclusions. These are: (i) a single nucleus, usually like a signet-ring; and (ii) a sling-like structure—the remains of buccal apparatus of the free flagellate.

Fig. 14. A living specimen of *Blastocystis hominis*, a vegetable organism very common in human stools. The living protoplasm forms a very thin granular layer surrounding an enormous more or less spherical inclusion of reserve material.

Fig. 15. Cyst of *Giardia* (= *Lamblia*) *intestinalis*, in iodine solution. The cysts of this flagellate usually contain 2 or 4 small nuclei—in this specimen 4—at one pole; and in addition rod-like and crescentic inclusions derived from the skeletal parts of the free form—axostyles and 'peristome' fibrils.



C. Dobell del.

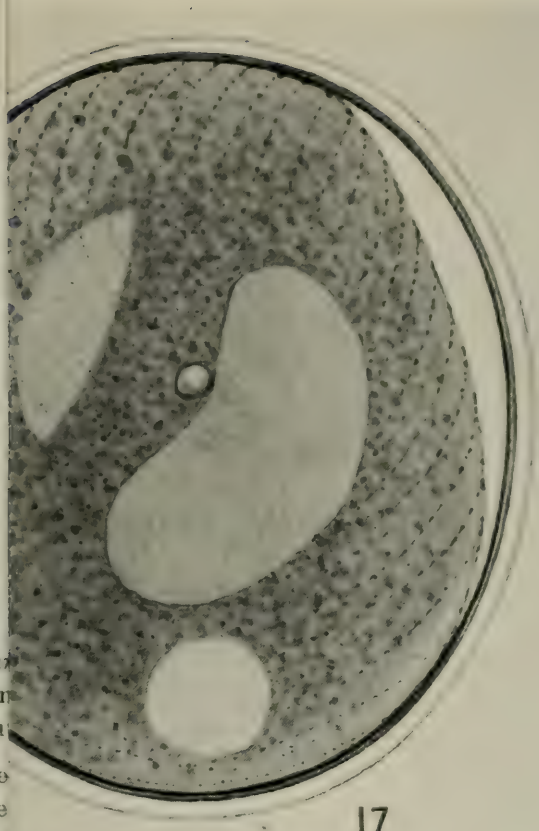


Fig. 16. *Bala*
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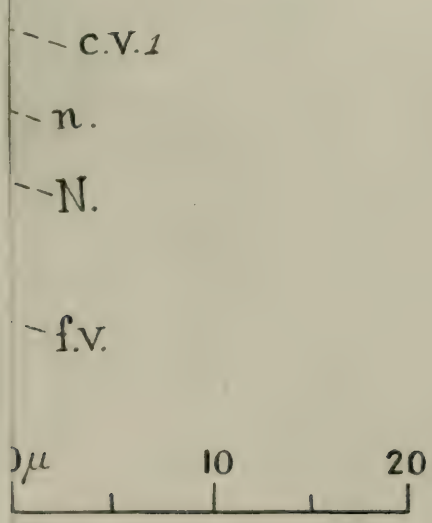
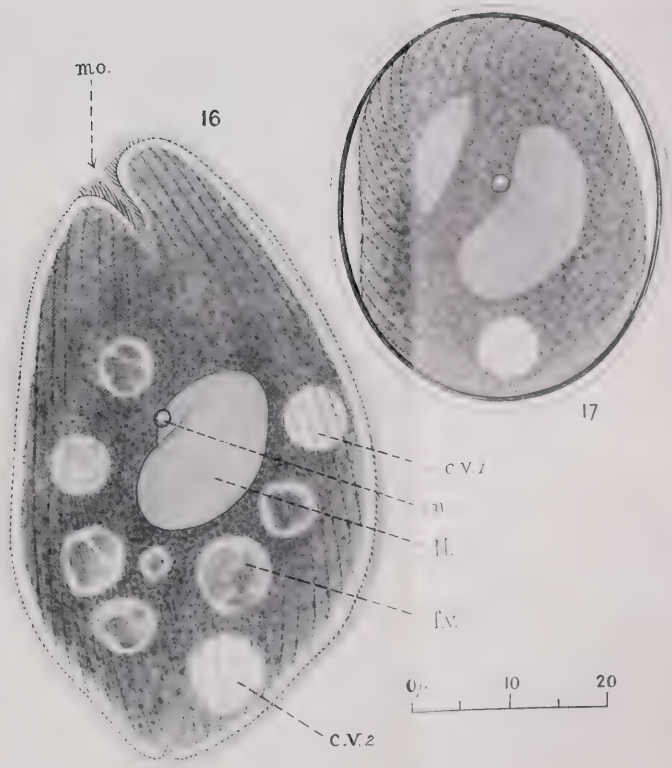


PLATE III

Fig. 16. *Balantidium coli*. Living animal. *N.*, meganucleus; *n.*, micronucleus; *c. v. 1*, anterior contractile vacuole; *c. v. 2*, posterior contractile vacuole; *f. v.*, food vacuole; *m.*, mouth. × 2,000.

Fig. 17. Recently formed cyst of *Balantidium coli*, at the stage of development usually seen in freshly-passed faeces. The nuclei, posterior contractile vacuole, and remains of ciliary investment are visible. × 2,000



C. Dobell del.

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MEDICAL RESEARCH COUNCIL

The Science of Ventilation and Open Air Treatment. Part II





LONDON
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1920

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MEDICAL RESEARCH COUNCIL

The Science of Ventilation and Open Air Treatment. Part II

BY

LEONARD HILL, M.B., F.R.S.

Director of Department of Applied Physiology
Medical Research Council

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PART II

CONTENTS

SECTION I

CHAP.		PAGE
I.	On Radiation	7
	Radio-active elements. Ionization of gases by the radio-elements. The electronic charge. Changes of the radio-elements. The nature of the α-, β-, and γ-rays	7
	The radio elements and the periodic table	11
	Arrangement of electrons in the atom	13
	Chemical and physical properties of elements	15
	Classification of substances on the electronic theory	16
	The motion and mass of electrons	16
	Radius of the electron	17
	Velocity and energy of electrons	17
	The electro-magnetic theory of light	18
	Influence of the medium on wave motion	20
	X-rays	22
	The photo-electric effect	23
	Phosphorescence and fluorescence	24
	The application of the electronic theory to physiology	25
	The relation between reflection, absorption, and transmission explained by the electronic theory	30
	Action of solar rays on life	31
	Energy curves of the sun and other sources of light	33
	The exchange of heat radiation	36
	Ionization of air	40
	Magnetic storms and Hertzian radiation	42
	The quantum theory	44
II.	The Penetration and Action of Light on Living Organisms	45
	The intensity of sunlight at altitudes, sea-level, and in cities	45
	The influence of sunlight on man	46
	The influence of light and dark on growth of animals	46
	The influence of high and low sun on the variability of plants	47
	The influence of extremes of temperature on the variability of animals	48
	The intensity of various sources of radiant energy	49
	Absorption and reflection of rays by the skin	49
	Physiological action of blue, violet, and ultra-violet rays and sensitization	50
	Absorption by pigment of the skin	52
	Protection of the eyes by tinted glasses	53
	Sun-burn	54
	Action of rays on the blood	55
	X- and γ-rays	56
III.	The Colour of the Skin and Racial Adaptation to Climate	57
	The effect of climate on the colour and texture of the skin	57
	The susceptibility of blondes and brunettes to climate and disease	60

(4521) Ps. 2781. Wt. 31723. 261/36. 2000. 7/20. O.U.P.

CHAP.		PAGE
	The adaptation of the negro	61
	Pigment in domestic animals and their adaptation to climate	62
	The white man in the tropics and infective disease	65
	The evolution of a mixed race adapted to tropical climates	67
IV.	Emission, Absorption, and Reflection of Light by White and Black Skin	69
	Leslie's classical experiments	69
	The emission of white and black skin	70
	The reflection of sunlight by white and black skin	71
	White and black skin temperatures	72
	The influence of pigment on the temperature of cold-blooded animals	74
V.	The Influence of Sunlight on Transpiration of Wet, Black, and White Surfaces	76
VI.	Sunlight in Tropical and Temperate Climates	81
VII.	Black Fur, Cheek, and Clothes Surface Temperatures	83
	The surface temperature of black fur as an indicator of radiant energy	84
	Comparison with the black bulb thermometer	87
	Absorption of white and coloured garments	88
	Absorption of white and coloured flowers	91
VIII.	Acclimatization to Insolation	92
	Experiments on animals	92
	The effect of coloured screens, wind, and diet	92
IX.	Body and Skin Temperature in the Tropics and the Effect of Exercise and Sweating	94
X.	Heat-Stroke	98
	The Black Hole of Calcutta	98
	Heat-stroke in moist heat	99
	The effect of breathing hot air	101
	Heat-stroke in dry hot air; the effect of wind	101
	The influence of the sun	102
	The influence of clothing	102
	Spinal pads and helmets	103
	Heat-stroke and unfitness from disease	104
	Air-temperature and heat-stroke	104
	Capacity of sweating mechanism	105
	Treatment of heat-stroke	105
	Water sprays and fans	106
	Pathology of heat-stroke	109
	Exhaustion of the adrenal glands	109
	The limits of temperature compatible with life of bioplasm	110
XI.	Food, Exercise, and Climate	111
	The over-heating effect of certain foods in the tropics	111
	The specific dynamic action of protein	111
	The influence of a sedentary and confined life in reducing metabolism	113
	The importance of an adequate supply of vitamines and of variety of protein food	114
	The effect of open-air exercise	120
XII.	Conclusions as to Colour of Skin and Climate	122

SECTION II

CHAP.		PAGE
I.	THE CHEMICAL PURITY OF THE ATMOSPHERE IN CROWDED CONFINED PLACES AND IN MINES	125
	The smell of crowded rooms	125
	The supposed existence of organic poison in exhaled air	127
	Ozone as a deodorizer	129
	Oxygen in crowded rooms and at high altitudes	132
	Effect of want of oxygen	135
	Carbon monoxide poisoning	137
	Carbon dioxide in crowded rooms	137
	Effect of breathing excess of carbon dioxide	138
	The atmosphere in submarines and divers' helmets	139
	The air of mines	141
II.	THE SMOKE NUISANCE AND THE ECONOMIC USE OF COAL	143
	The damage done by smoke	143
	The deposit of soot in cities and country districts	144
	The number of particles in the air	144
	Effect of smoke on plants	145
	Relation of deaths from bronchial disease to fogs	146
	Gas heating and smokeless fuel	147
III.	DUST	150
	Methods of estimation	150
	Dust in city and country, in places of business and factories	152
	The influence of dust on pulmonary disease	155
	Bacteria in air of crowded places	156
	Silicosis	158
	Pulmonary tuberculosis and dusty occupations	162
	Benign action of coal dust	165
IV.	HEAT STAGNATION AND FITNESS	168
	The effect of warm stagnant air	168
	Working efficiency and body heat stagnation	169
	The effect of heat stagnation on the circulation	170
	The effect of heat stagnation on the internal secretions	179
V.	HEALTH AND ENVIRONMENT	181
	Climate and health	181
	Recent change in national habits of life	181
	Expectation of life, how influenced in the last fifty years	181
	Mortality in city and rural districts	182
	Density of population and the death rate	183
	The fitness of recruits medically examined 1917–18	183
	The effect of industrialism on women during the war	183
	Incidence of tuberculosis in certain occupations	187
	Tenement dwellings	188
	Tuberculosis of youth, middle-age, and old-age	195
	Sterility and city life	196
	The influence of diet in cities, want of vitamines	196
	Infant mortality	198
	The need for garden cities and outdoor exercise	202
VI.	FEVER AND OPEN-AIR TREATMENT	203
	The heat lost and produced in fever	203
	The treatment of fever by open air	205
	Sanatorium treatment	206

CHAP.		PAGE
VII.	Treatment at High Alpine Attitudes	210
VIII.	Concerning Clothes	214
	Thermal conductivity of clothing materials	218
	Permeability of clothing materials	218
	Protective power of clothing materials	220
	Protection from insolation by clothing materials	224
	Emission of heat by clothing materials	226
	Hygroscopic property of clothing materials	228
	Heat evolved by dry clothing materials on taking up water	233
	Thermal conductivity of wet clothing materials	236
	Measurement of heat-retaining power of clothing materials	237
	Tropical clothing	245
	Heat-retaining power of wet clothes	247
	Trench feet and frigorism	251
IX.	Methods of Ventilation and Heating	256
	Fumes from imperfect combustion	256
	Humidity and comfort	257
	Ill-ventilated places	257
	Cooling power of atmosphere and efficiency of workers	260
	Carbon dioxide a measurement of ventilation	263
	Ventilation of railway cars	264
	The rate of heat loss by walls of submarines, dug outs, and chambers occupied by men	266
	Heat production of illuminants	274
	Effect of sun on warmth of buildings	274
	Chief systems of ventilation and heating	275
	Average temperature of rooms in England and U.S.A.	277
	Fan ventilation, its direction of importance	278
	Ventilation of engine rooms, &c., by 'joy wheels'	279
	Need for skilled control of ventilation	280
	The plenum system	282
	Ventilation of tropical houses	286
	Economics of heating systems	287
	The open fire	287
	Measurement of radiant energy	288
	A grate suitable for burning coke	291
	The maintenance of a comfortable cooling power in a room heated by a fire	292

Section I

CHAPTER I

On Radiation.[1]

It is argued that the energy of the universe is a constant quantity, convertible, but not gained or lost, and that free energy (free to do work) is always striving towards a minimum, becoming dissipated as heat. While the forms of energy now recognized are many, it seems likely that in the immediate future the connexion between these different forms will be more clearly understood.

More than half a century ago the study of electrolysis led Faraday to feel the identity of chemical and electrical energy. Radiation has become part of electrical science, and it appears probable that the inertia of matter may be attributed to electrical properties.

Before considering radiation in detail and its biological effect we may review the present state of knowledge concerning the constituent parts of matter which are now recognized to be the cause of all radiations.

The definition which stated that a molecule was the smallest particle of matter which could exist in the free state, and the atom the smallest constituent part of the molecule, has now been replaced by the generally accepted theory that an atom consists of a positively charged nucleus surrounded by negatively charged particles known as electrons. Practically the whole of the atomic mass is concentrated in the positive nucleus. Under certain circumstances the electrons can be separated from the parent atom and their properties studied.

The radio-active elements. The atomic structure has been partly revealed by a class of substances known as the radio-active elements. The characteristic of these elements is that they undergo spontaneous atomic transformations, i.e. that without any external force acting on the atom it becomes transformed into a new substance. This transformation must consist of an addition or loss to the existing atom or a rearrangement of the parts. It is found to be due to the second of these causes (also possibly the third), for by suitable means the part given off may not only be detected but some of its properties discovered. This emission consists of three kinds of rays, known as α-, β-, and γ-rays respectively. The α-rays carry a positive charge, the β-rays a negative one, and the γ-rays are uncharged.

Ionization of gases by the radio elements. When these rays are allowed to pass through a gas they are able to knock electrons off the gas atoms. An atom is in the ordinary state electrically neutral, and therefore the total negative charge of the electrons is equal to the positive charge of the nucleus. When an electron gets knocked off, therefore, it follows that the remainder of the atom is positively

[1] I am indebted to Miss D. Hargood Ash for a revision of the physics in this chapter and in particular for help in the statement of the electronic theory.

charged, while if the electron becomes attached to another atom this will be negatively charged. Either of these charged bodies may collect a group of molecules, and in this state is known as an ion.

Following up the work of J. J. Thomson, C. T. R. Wilson, and H. A. Wilson, R. W. Millikan showed that very small oil droplets, suspended in ionized air, continually picked up ions. The oil droplet (which was in general found to be charged by the frictional process involved in blowing the spray) was blown between two plates which

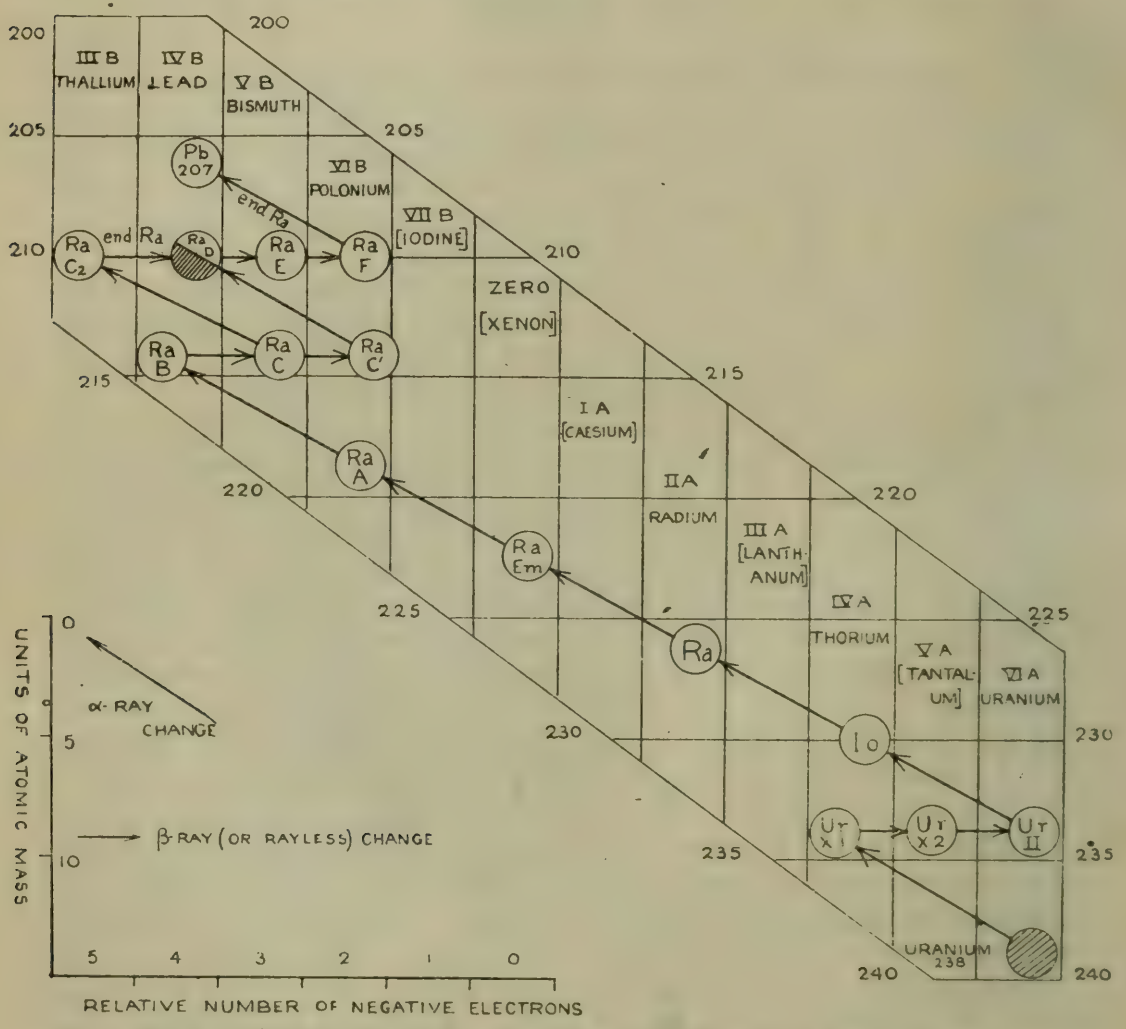


Fig. 1. Transformation of Uranium through Radium to lead. Soddy.

could be charged, the one positively and the other negatively. The droplet fell under gravity when the plates were uncharged, but just before it reached the lower one they were charged, the charge being so arranged that the electrical force acting upwards on the charged droplet was a little greater than the gravitational force acting downwards upon it, and the droplet travelled upwards. By thus charging and discharging the plates the droplet could be kept moving up and down for as long as desired. It was rendered visible by ultra-microscopical illumination, and the time taken for each upward and downward journey noted. When the droplet caught an ion the upward rate of movement (depending upon the electrical charge on the droplet) was altered and the capture detected.

The electronic charge. Millikan was able by this method and by suitably adjusting the electrical field to obtain the value of the charge on the drop, and found that it was always made up of a multiple of a fundamental charge. Not only this, but that the initial frictional charge was also a multiple of the fundamental charge. Since this charge was also the one added by a capture he concluded it was the ionic charge, or the charge carried by an electron, and found its value to be $4{\cdot}774 \times 10^{-10}$ electrostatic units.[1] Millikan's experimental method was so delicate that he was able to weigh accurately to one ten billionth of a milligramme!

Changes of the radio elements. Even when the charge of the electron is known we are still in ignorance of the number and arrangement of the constituent parts of the atom. The study of the changes of the radio elements has, however, yielded a certain amount of information on this point. As already mentioned, the radio elements are characterized by their power of undergoing spontaneous disintegration, that is to say one radio element may become transformed into another. The best known example of this is the changes which the radio element radium undergoes, this element giving off rays and eventually becoming lead. It is found, however, that radium is not the original element but is a product of the radio element uranium.

Fig. 1, taken from Soddy's *Radio Elements*, Part II, 1914, shows graphically the transformations which uranium undergoes in becoming radium and from radium in becoming lead. The substances which uranium becomes for various periods during this transformation, in the order indicated by Fig. 1, are as follows:

Uranium.	Radium B.
Uranium X 1.	Radium C.
Uranium X 2.	Radium C 1, Radium C 2.
Uranium II.	Radium D.
Ionium.	Radium E.
Radium.	Radium F.
Radium Emanation.	Lead.
Radium A.	

The long slanting arrows indicate the loss of α-rays, the short horizontal ones the loss of β-rays. Other radio elements which present similar series of changes are actinium and thorium.

The change from one element to the next in the series takes widely different times, but in each case the amount existing varies with the time according to the exponential law, which may be stated by saying, that the rate of change (or decay) of the substance is proportional to the amount of substance remaining. A well-known instance of this law is met with in Newton's law of cooling, which states that the rate of cooling of a body is proportional to the excess of the temperature of the body over the temperature of the surroundings. This is familiar to every one in the fact that a cup of tea quickly loses its 'hotness', but cools very slowly as its temperature approaches that of the room. Fig. 2, taken from Rutherford's *Radio-active Substances and their Radiations*, gives the decay of uranium X, and

[1] An electrostatic unit charge of electricity is that positive charge which repels an equal positive charge, distant one centimetre away, with a force of one dyne.

shows that the activity (measured by the rays given off, and proportional to the amount of substance present) has fallen to half its initial value in 24·6 days, and that it has not fallen to zero at the end of 150 days. For this reason it is the half transformation period which is usually stated in radio-active transformations. This half transformation period for uranium is 5×10^9 years, for radium 2000 years, for radium A only 3 minutes. The value of the average life of the atom (by which is meant the average length of time which any atom on entering one state may expect to remain in that state before becoming transformed into the next of the series) is deduced mathematically from the known rate of decay of the element by the laws of probability, and in the three cases given above is $7{\cdot}2 \times 10^9$ years, 2880 years, and 4·32 minutes respectively (Rutherford).

The nature of the α-, β-, *and* γ-*rays.* It was Mme Curie who first showed that α-rays consisted of charged particles, but it was not till 1903

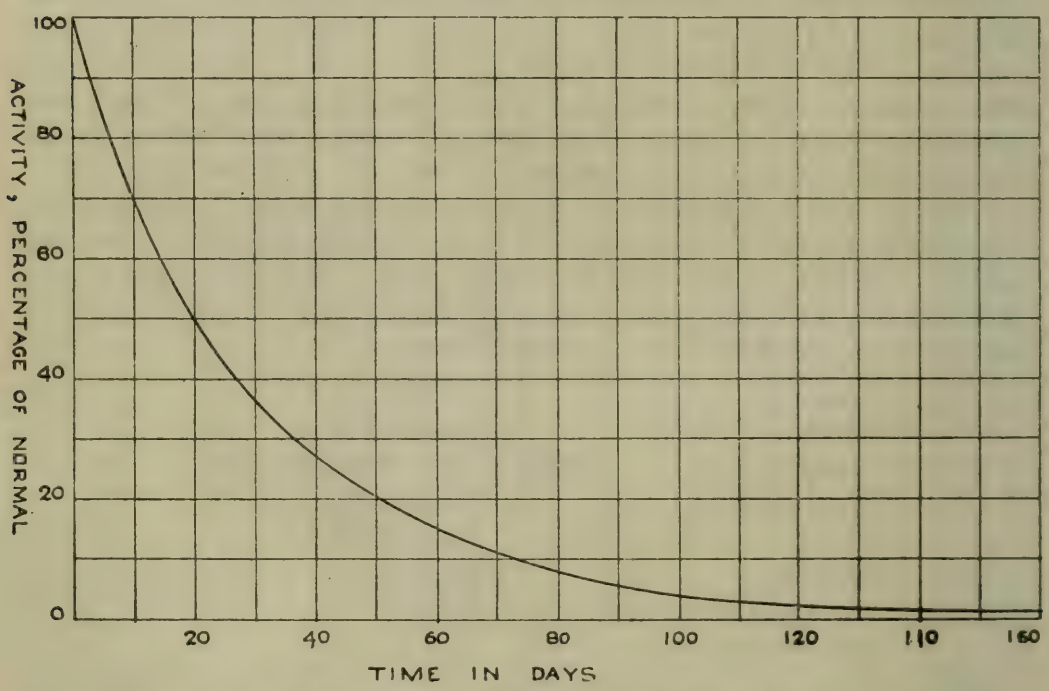


Fig. 2. Decay of Uranium X. Rutherford.

that Rutherford was able to deflect them both by electric and magnetic fields, thus showing that these particles were positively charged. Rutherford and Geiger found the value of this charge to be $9{\cdot}3 \times 10^{-10}$ electrostatic units. Since this value is just double that obtained for the electronic charge, it was concluded that the α-particle carried two positive charges. Rutherford and Royds were then able to demonstrate experimentally that this charged particle was actually a charged Helium atom. The negatively charged β-rays have been found to consist of electrons, and the γ-rays which appear uncharged are like the X-rays, which are produced in a vacuum tube when the cathode rays due to an electric discharge in the tube fall on a metal.

The radio-active emanation which is also given off by radium is radio-active matter in process of transmutation, and quickly decaying in radio-active power by the emission of α-rays.

The radio elements and the Periodic Table. A characteristic of

every known element is its atomic weight. If the elements are arranged in the order of their atomic weights (with one or two exceptions) as in the well-known Periodic Table, Fig. 3, they form a series the properties of which recur periodically. These periodic recurrences are shown in the vertical lines of the Table: for example, Group O contains the inert gases Helium, Neon, Argon, &c.; Group I the alkali metals, Lithium, Sodium, Potassium, &c., and so on. The elements considered along the horizontal lines of the Table show gradually increasing properties. One of the most marked of these properties is the increase in valency. The Group O elements have no valency, those in Group I are monovalent, those in Group II divalent, those in Group III trivalent, and so on, the halogen elements in Group VII having a valency of seven. By valency is meant the charge carried by an ion in solution, that is to say, when certain substances form solutions they become dissociated into two parts, one of which is negatively and the other positively charged; these are termed cath- and an-ions respectively (the ion in this case is not necessarily identical with the gaseous ion already mentioned), the ion being monovalent, divalent, and so on, according to the number of charges which it carries. The valency of an element also determines the nature of its chemical compounds.

When a radio element loses an α-particle or helium atom it would be expected that its atomic weight would decrease by the atomic weight of helium, that is to say by four units. This is what is found to happen, and that the element at the same time moves two places to the left in the Periodic Table. Soddy has pointed out that when the element loses a β-particle or electron, the element moves one place to the right, but in this case the atomic weight is unaltered. In both these cases the atom possesses the valency appropriate to the Group in which it is. If, for example, the element were initially in Group IV and therefore tetravalent, it would on losing an α-particle be in Group II, and therefore divalent; if then by losing a β-particle it moved to Group III, it would become trivalent; further, if it then lost another β-particle, it would be back in Group IV from which it started. Soddy, to whose work the knowledge of this part of the subject is largely due, was thus led to the conclusion that two substances with different atomic weights could be identical in chemical character and occupy the same place in the Periodic Table. Such elements he termed isotropic, or isotropes.

Soddy therefore regards the place of an element in the Periodic Table to be determined not by its atomic weight but by the relative number of positive and negative charges which it contains, it being necessary to suppose that there are at least two sets of electrons in the atom, and that it is the inner or nuclear electrons which affect the position of the element in the Periodic Table, and therefore are those which are ejected in radio-active changes.

These changes for the element uranium are clearly brought out in Soddy's diagram Fig. 1. The ordinates represent units of atomic weight, and the abscissae the number of successive places in the Periodic Table corresponding to the electrical constant (or relative number of positive and negative charges in the nucleus) of the atom. In passing from right to left of the diagram, each place corresponds

THE ELEMENTS, THEIR ATOMIC NUMBERS, ATOMIC WEIGHTS, AND CHEMICAL POSITIONS

1 H
1·008

0	I	II	III	IV	V	VI	VII	VIII
2 He 3·99	3 Li 6·94	4 Be 9·1	5 B 11·0	6 C 12·00	7 N 14·01	8 O 16·00	9 F 19·0	
10 Ne 20·2	11 Na 23·00	12 Mg 24·32	13 Al 27·1	14 Si 28·3	15 P 31·04	16 S 32·06	17 Cl 35·46	
18 A *39·88*	19 K *39·10*	20 Ca 40·07	21 Sc 44·1	22 Ti 48·1	23 V 51·0	24 Cr 52·0	25 Mn 54·93	26 Fe 27 Co 28 Ni *55·84* *58·97* *58·68*
....	29 Cu 63·57	30 Zn 65·37	31 Ga 69·9	32 Ge 72·5	33 As 74·96	34 Se 79·2	35 Br 79·92	
36 Kr 82·92	37 Rb 85·45	38 Sr 87·63	39 Y 88·7	40 Zr 90·6	41 Nb 93·5	42 Mo 96·0	**43**—	44 Ru 45 Rh 46 Pd 101·7 102·9 106·7
....	47 Ag 107·88	48 Cd 112·40	49 In 114·8	50 Sn 118·7	51 Sb 120·2	52 Te *127·5*	53 J *126·92*	
54 X 130·2	55 Cs 132·81	56 Ba 137·37	57 La 58 Ce 59 Pr 60 Nd 61–62 Sm 63 Eu 64 Gd 65 Tb 66 Ds 139·0 140·25 140·6 144·3 150·4 152 157·3 159·2 162·5					
67 Ho 68 Ev 69 Tu 70 Yb 71 Lu **72**— 163·5 167·7 168·5 173·5 175·0					73 Ta 181·5	74 W 184·0	**75**—	76 Os 77 Ir 78 Pt 190·9 193·1 195·2
....	79 Au 197·2	80 Hg 200·6	81 Tl 204·0	82 Pb 207·20	83 Bi 208·0	84 Po (210·0)	**85**—	
86 Em (222·0)	**87**—	88 Ra 226·0	89 Ac (227)	90 Th 232·15	UrX2 (234)	92 Ur 238·2	....	

Elements the atomic numbers of which are not in the order of atomic weights are in italics. The numbers corresponding to missing elements are in bold-faced type.

1 Hydrogen
2 Helium
3 Lithium
4 Beryllium
5 Boron
6 Carbon
7 Nitrogen
8 Oxygen
9 Fluorine
10 Neon
11 Sodium
12 Magnesium
13 Aluminium
14 Silicon
15 Phosphorus
16 Sulphur
17 Chlorine
18 Argon
19 Potassium
20 Calcium
21 Scandium
22 Titanium
23 Vanadium
24 Chromium
25 Manganese
26 Iron
27 Cobalt
28 Nickel
29 Copper
30 Zinc
31 Gallium
32 Germanium
33 Arsenic
34 Selenium
35 Bromine
36 Krypton
37 Rubidium
38 Strontium
39 Yttrium
40 Zirconium
41 Niobium
42 Molybdenum
43 ——
44 Rhuthenium
45 Rhodium
46 Paladium
47 Silver
48 Cadmium
49 Indium
50 Tin
51 Antimony
52 Tellurium
53 Iodine
54 Xenon
55 Caesium
56 Barium
57 Lanthanum
58 Cerium
59 Praseodymium
60 Neodymium
61 ——
62 Samarium
63 Europium
64 Gadolinium
65 Terbium
66 Dyprosium
67 Holmium
68 Erbium
69 Thulium
70 Ytterbium
71 Lutecium
72 ——
73 Tantalum
74 Tungsten
75 ——
76 Osmium
77 Iridium
78 Platinum
79 Gold
80 Mercury
81 Thallium
82 Lead
83 Bismuth
84 Polonium
85 ——
86 Emanation
87 ——
88 Radium
89 Actinium
90 Thorium
91 Uranium X_2
92 Uranium

Fig. 3. Periodic table after Millikan 'The Electron'.

with an increase in the electrical content of the atom by one unit of negative electricity or with a decrease of positive electricity by one unit of charge. It will be seen from the diagram that in the uranium series considered the following are isotropic:

Group VI. A Uranium I and Uranium II.
„ IV. A Uranium X and Ionium.
„ VI. B Polonium (or Radium F) and Radium A.
„ IV. B Lead, Radium B and Radium D.

It is seen from the above that the radio elements become transformed by the loss of a helium particle, and each element differs from the next by the same unit charge; it is therefore reasonable to suppose that all elements may be built up of one substance. Hydrogen being the lightest known element naturally suggests itself as this fundamental substance. The fact that Rutherford[1] was apparently able to bring about the expulsion of hydrogen atoms from nitrogen gives some support to this hypothesis. He bombarded nitrogen molecules with α-particles from radium, and detected the hydrogen atoms produced by the scintillation method, using a zinc sulphide screen.

By means of a new method of positive ray analysis F. W. Aston[2] has obtained measurements of mass and other evidence which, he says, proves that carbon, oxygen, nitrogen, hydrogen, and helium are 'pure' elements, neon, argon, chlorine, mercury, and krypton are mixed consisting of two isotropes in the case of neon, at least two in the case of chlorine, at least three or four in the case of mercury, and six in the case of krypton.

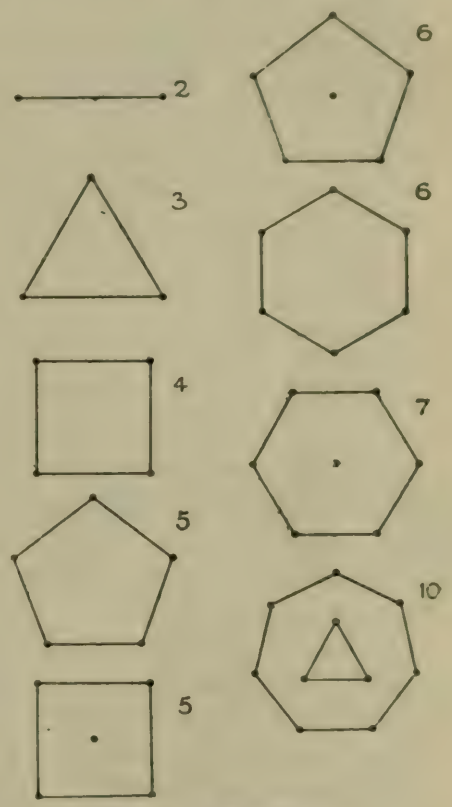


Fig. 4. After Mayer.

Arrangement of electrons in the atom. The general arrangement of the electrons in the atom is not at present known. It is supposed, however, that they are arranged on concentric spherical shells, or rings, those in the inner rings being concerned with the atomic properties, while those in the outer rings would seem to be less closely attached to the nucleus and to determine the chemical and many physical properties. Mayer has shown experimentally by means of magnets how similar charges free to move will place themselves with respect to a charge of opposite sign. He magnetized a number of needles to

[1] *Phil. Mag.*, 1919, **37**, 537. Atoms of mass 3, carrying two positive charges, were also expelled, probably an isotrope of helium. These alone were expelled from oxygen. Helium consists of four hydrogen nuclei and two binding electrons, the isotrope of three and one. Bakerian Lecture, R. S., June 8, 1920.

[2] *Nature*, Dec. 18, 1919, and Mar. 4, 1920.

the same strength, and floated them in water by sticking them through pieces of cork. All the poles of one sign were upwards and sticking out of the water. The controlling charge was the pole of a magnet suspended a little above the needles. The accompanying diagrams and figures show some of the results he obtained. It will be noticed that the magnets took up positions in rings of different numbers.

The figures show the arrangements of magnets obtained when the innermost group consisted of 1, 2, 3, 4, and 5 magnets respectively, the number of concentric groups being II, III, IV. Fig. 4 represents some of these arrangements, for example No. 10 represents II 3.

	1	2	3	4	5
II	1. 5 1. 6 1. 7	2. 6 2 7	3. 7 3. 8	4. 8 4. 9	5. 9
III	1. 5. 9 1. 6. 9 1. 6. 10 1. 6. 11	2. 7. 10 2. 8. 10 2. 7. 11	3. 7. 10 3. 7. 11 3. 8. 10 3. 8. 11 3. 8. 12 3. 8. 13	4. 8. 12 4. 8. 13 4. 9. 13	5. 9. 12 5. 9. 13
IV	1. 5. 9. 12 1. 5. 9. 13 1. 6. 9. 12 1. 6. 10. 12 1. 6. 10. 13 1. 6. 11. 12 1. 6. 11. 13 1. 6. 11. 14 1. 6. 11. 15	2. 7. 10. 15 2 7. 12. 14	3. 7. 12. 13 3. 7. 12. 14 3. 7. 13. 14 3. 7. 13. 15	4. 9 13. 14 4. 9. 13. 15 4. 9. 14. 15	

J. J. Thomson suggests that the electrons of an atom group themselves in somewhat this way, and that while some arrangements are stable others are not. If the electrons are in unstable arrangement the atom will readily part with or attract electrons, if by so doing it becomes more stable.

It has already been stated that ions in solution carry positive and negative charges which determine their valency. Now an atom is ordinarily electrically neutral, and therefore the total electronic charge must be equal to that of the nucleus. It would therefore seem that when a molecule of a substance becomes dissociated in solution one of the atoms must lose one (two or three, &c.) electrons, and so become positively charged, while the other atom must take hold of this electron and become negatively charged. In these cases the atom that loses the electron may be supposed more stable without it, while its addition to the other atom may cause that arrangement also to be more stable. The electrons that can thus be exchanged are supposed to be the outer ones and are known as valency electrons.

Soddy has shown that it is the number of inner or nuclear electrons with respect to the number of positive charges that controls the valency, and therefore the number of valency electrons.

To make this clearer suppose that in a certain stable arrangement there are N positive charges, then there will be also N electrons. If n of these are valency electrons there will be $N-n$ inner or nuclear electrons. Suppose this is the arrangement of an element in Group O of the Periodic Table (as these elements have no valency they may be

considered stable), for example argon, then since, as already shown, the next element potassium possesses one more positive charge, i. e. $N+1$, the total number of electrons must be $N+1$, and if the number of nuclear electrons is $N-n$ as before, then the number of valency electrons will be $n+1$, that is to say, the valency will have increased by one, as is found to be the case, and the relative number of positive and negative charges in the nucleus will be increased by one positive charge. The odd electron in this case causes instability or is easily parted with. For the next position of stability the number of nuclear electrons may be supposed increased. Therefore, though it is the valency electrons which actually take part in chemical change, it is the electrical charge of the nucleus which primarily controls the power of the ring to lose or gain electrons.

Chemical and physical properties of elements. To explain exactly how the chemical and physical properties of the elements are controlled by the valency electrons a large number of hypotheses have been put forward. Suppose the electrons to be at rest relative to the nucleus, and that the atom consists of a central positive charge and a single external electron. As the two charges are separated by a finite distance they will behave like a short bar magnet and exert an electric field which will fall off inversely as the cube of the distance from the point midway between them.

J. J. Thomson postulates the existence of two kinds of molecules, to both of which this magnet idea may be applied ; those in which the individual atoms bear a charge (each atom must here be considered a pole of the magnet), and those in which the individual atoms are uncharged. He thus divides compounds into two classes :

(i) Those in which the forces between the atoms are not sufficient to bring about the passage of an electron, i. e. the individual atoms are uncharged.

(ii) Those in which such an exchange does take place. Molecules of this kind he terms ionized.

In the first class, of which hydrogen may be taken as an example, it is supposed that a Faraday tube of force starting from an electron of one atom ends on the positive nucleus of the other atom, the tube starting from the second atom ending on the positive charge of the first, and the atoms thus being bound together. Hydrochloric acid may be taken as an example of the second class. It can be supposed that an electron from the hydrogen atom (it will be remembered that hydrogen has one valency electron) attaches itself to a chlorine atom ; the former will thus become positively and the latter negatively charged, and the mutual attraction of the opposite charges will hold the two atoms together. In this way the chemical actions of compounds may be explained. The physical properties such as surface tension, latent heat, &c., may also be explained, by supposing in Class I that the individual atoms of the molecules act as magnets and attract other molecules ; in Class II that each atom acts as a pole, and the whole molecule as a magnet, and so attracts other molecules. In Class II since the poles will be further apart than in Class I (the force exerted by a magnet varies directly with the distance between the poles) the attraction between the molecules should be greater. The theories of Abegg and Stark offer somewhat similar explanations of Valency and

chemical combination, and J. Langmuir[1] has recently put forward a comprehensive theory based upon the chemical properties of the elements as shown in the periodic table.

Classification of Substances on the Electronic Theory. N. R. Campbell divides the various properties of substances into three classes:

I. Properties which depend neither on the nature of the atoms nor on the relation between them, for example, the gravitational constant, electronic charge, &c.

II. Properties which depend only on the number and nature of the atoms in the substance, but not on their state of chemical combination or on the physical state of the substance. For example mass.

III. Properties which are not only determined by the number and nature of the atoms in the substance, but depend also on their state of chemical combination and physical aggregation. For example, chemical properties, mechanical properties (elastic constants, melting and boiling points, surface tension, and so on).

The well-known fact of two metals forming an alloy with a melting point less than that of either of the constituents illustrates well the behaviour of substances included in Class III.

The motion and mass of electrons. So far the electron has been considered at rest with respect to the nucleus of the atom, but as there is an attraction between the two, the electron must have a motion to prevent being drawn into the nucleus, it must in fact revolve in some sort of orbit in an analogous manner to that in which the planets of the solar system revolve round the sun. Little is known about the orbits of electrons or their velocities, but a certain amount of knowledge with respect to the latter may be deduced from the disintegration of the radio elements. It has already been stated that during this disintegration the radio elements give off β-particles which are identical with electrons. Perhaps the most surprising result from this study of the β-particles is found in the consideration of its mass. It can be shown theoretically that a moving charge possesses magnetic energy, the amount of which is given by $e^2\ w^2/3a$, where e is the charge of electricity moving with a velocity w, the charge being spread over a sphere of radius a. Therefore an electron of mass m and radius a moving with a velocity w would have in addition to its kinetic energy $\frac{1}{2}\ mw^2$ this magnetic energy, the sum of the two energies being $\frac{1}{2}\ mw^2 + \frac{e^2\ w^2}{3a}$, which by putting $2e^2/3a$ equal to m_1 may be written $\frac{1}{2}\ w^2(m + m_1)$, that is, the total energy is the same as that which would have been obtained if a mass m_1 equal to $2e^2/3a$ had been added to the original mass m of the electron.

The above formula for the magnetic energy holds only for speeds small compared with that of light, 3×10^{10} cm. per sec. It was found theoretically that the electrical mass $2e^2/3a$ should increase with the increase of velocity. To see if this were the case experiments were carried out using β-particles from radium which travel with velocities varying from three-tenths up to ninety-eight-hundredths that of light. Bucherer found that the rate of variation of the mass of the electron with its speed agreed with the theoretical variation of the electrical

[1] *J. Am. Chem. Soc.*, June, 1919.

mass with speed, or in other words that the whole mass of the electron was due to its charge! This fact is at once suggestive that all mass is electrical in origin. 'The two entities,' writes R. W. Millikan, 'electricity and matter begin to look like different aspects of the same thing.'

Radius of the electron. The velocity w and the ratio of the charge e to the mass m of a charged particle may be experimentally determined by deflecting the particle with magnetic and electrical fields. If the charge e is known, m is easily calculated. One method for the determination of e has already been indicated (p. 9). Since also the electrical mass is equal to $2/3\ e^2/a$ where a is the radius of the sphere over which the charge is spread, and in the case of the electron this is found equal to its whole mass m, the radius can be determined, and is found to be 2×10^{-13}, or but one-fifty-thousandth of the radius of the atom (10^{-8} cm.). If the mass of the positive nucleus be taken as wholly electrical, for which, however, there is no experimental evidence, the value of a is only 1/2000 of the value for the electron, since it is nearly 2,000 times heavier (Millikan).

Velocity and energy of electrons. When α- and β-particles are emitted from an atom by the disintegration of the radio elements they travel with great speeds, that of the β-particles varying from about 10^{10} to 3×10^{10} cm. per sec., that of the α-particles being about $0{\cdot}2 \times 10^{10}$ cm. per sec. It is these particles moving with high velocity which knock the electrons off the gas atoms and cause the ionization of gases already referred to. C. T. R. Wilson has photographed the track of both α- and β-particles in air, the path being marked by the droplets of water which condense on to the ions. The photographs show very plainly the much greater ionizing power of the α-particle which is massive and slow moving when compared with the β-particle. They also indicate that an α-particle may pass right through a gas molecule without doing any damage beyond occasionally knocking off an electron, and without being deflected from its path.

The electron, when part of the atom, possesses an enormous store of energy, but how the energy is stored or how let loose is unknown. According to Oliver Lodge the α-particle is 400 million times more energetic than a rifle bullet travelling with 1/15 the speed of light. The atoms of matter then hold a colossal store of energy. Lodge says that the atomic energy of a pound of matter would suffice to lift the German fleet from Scapa-flow and pile it on Mount Nevis. Soddy writes: 'The primary sources of natural energy by virtue of which the universe keeps going over immense periods of time are to be sought not in the great masses of glowing matter dotted about the heavens, nor in their motions under the action of gravity, nor in any of the grosser relations between energy and matter in bulk, but in the individual atoms of which it is made up. Radio-activity alone, including in that term processes involving atomic transformations, is competent to be regarded as the mainspring of the universe. The radio elements may not be alone in parting with their stores of internal energy, radio-activity may be merely one type which has been recognized of changes which in greater or less degree may be proceeding in all matter.' There is an evolution and ceaseless change, a birth and death in the non-living no less than in the living substance. A hap'orth of electric current

purchased to light an incandescent lamp contains more electrons, says Millikan, than the population of Chicago could count if at it day and night for 2,000 years, an estimate which gives us some conception of a universe infinitely little such as the heavens afford of one infinitely great.

> Look how the floor of heaven
> Is thick inlaid with patines of bright gold.
> There's not the smallest orb which thou behold'st
> But in his motion like an angel sings,
> Still quiring to the young-eyed cherubins.
> Such harmony is in immortal souls;
> But whilst this muddy vesture of decay
> Doth grossly close it in, we cannot hear it.

The electro-magnetic theory of light. It is well known from elementary electrical and magnetic theory that the electrical intensity at any point due to a charge e at a distance d from the point is $e/\kappa d^2$, where κ is constant for any one medium, but varies for different media. Similarly the magnetic intensity due to a pole of strength m is given by $m/\mu d^2$, where μ is constant for any one medium but varies for different media. κ and μ are known as the 'specific inductive capacity' or 'dielectric constant' and the 'magnetic permeability' respectively of the substance. Clerk Maxwell showed mathematically that when an electro-magnetic disturbance was produced at one point the change in electric intensity at a point a distance from it was not instantaneous as was formerly supposed, but that the effect of the disturbance took a finite time to travel. He showed, in fact, that electrical intensity was propagated by wave motion with a finite velocity. This velocity v was given by the relation

$$v = \frac{c}{\sqrt{\kappa\mu}},$$

where κ and μ are the specific inductive capacity and the magnetic permeability of the medium and c is a constant for all media. For air κ and μ may be taken as equal to 1, and therefore the velocity in air should be numerically equal to the constant c. Maxwell showed in 1865 that this value for the velocity was 3×10^{10} cm. per second, and pointed out the fact that this was the same value as that obtained experimentally for the velocity of light in air! Maxwell's theory was later verified by the electrical experiments of Hertz.

It can be shown that charged molecules or atoms, or electrons oscillating, would cause rapidly alternating electro-magnetic fields—that is to say, would cause a disturbance similar to that considered by Maxwell, and which would therefore travel with the speed of light. It is also found that most of the known phenomena of light may be easily explained by supposing the light to be propagated by wave motion. The theory of the propagation of light by wave motion (apart from its electro-magnetic origin) was first stated in definite form by Huygens in 1678 and was eventually universally accepted in preference to its rival the corpuscular theory (which supposed small particles to be ejected from the source of light and to hit the retina of the eye and so cause the sensation of light) supported by Newton.

Recently the corpuscular theory of light has been revived in new form and support sought for it by some from the proof of the gravitational influence of the sun in deflecting light coming from the stars which passed close to the sun's rim, a proof determined by photography in the eclipse of 1919.

In considering radiation of all kinds the terms involved by the idea of wave motion must be frequently used and it will be as well to recall what is meant by them.

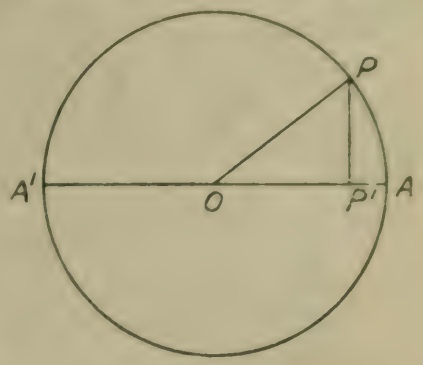


Fig. 5.

Suppose a point P, Fig. 5, moves round a circle with a constant velocity v. Let A′ OA be any diameter of the circle. If a line be dropped from P meeting the line A′OA in P′ so that the angle PP′O is a right angle, then keeping this angle a right angle the point P′ will move up and down the line A′OA with simple harmonic motion as P goes round the circle. It is a motion of this description which produces the simplest waves. If a curve is plotted with the displacements of the point P′ from O as ordinates and the times as abscissae a curve such as is shown in Fig. 6 will be obtained. The whole time taken by the point to go from O to A, back to A′, and to O again is called the period. A wave length is the curve included in one period,

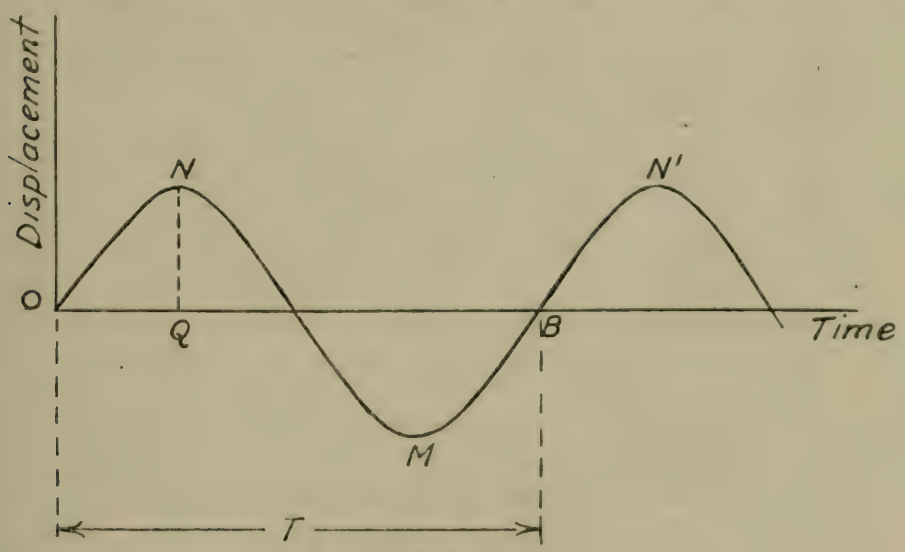


Fig. 6.

i. e. the length OB in Fig. 6. The amplitude of vibration is QN in Fig. 6, corresponding to OA in Fig. 5, and is the maximum displacement of the point P′ from O. Two waves are said to be in the same phase if their displacements are equal. For example, if the displacements of two similar waves can both be represented by the point N, Fig. 5, at the same time they are in the same phase, or if one is represented by N and the other by N′ they are in the same phase. If, however, one can be represented by N and the other by M they differ by half a wave length in phase, and so on. The number of times that the point P, Fig. 5, moves round the circle, or that P′ makes a complete oscillation

in a second, is known as the frequency and is usually denoted by n. The period T is clearly equal to the time taken for P to make one complete revolution, and is therefore equal to $1/n$. It is apparent that the wave length is the distance which any given condition of the displacement has travelled before the point P′, Fig. 5, comes to the same stage of its oscillation again, i. e. the distance it has travelled while P makes a complete revolution, which will be given by the velocity of P multiplied by the periodic time, or if λ is the wave length

$$\lambda = v\mathrm{T}$$
$$\text{or since } \mathrm{T} = 1/n$$
$$\lambda = v/n$$
$$\text{or} \qquad v = n\lambda,$$

which is the fundamental equation of wave motion connecting the velocity of propagation, the wave length, and frequency.

It follows from the above that if the velocity is constant, the greater the frequency the shorter the wave length.

Influence of the medium on wave motion. When a disturbance is caused by a vibrating body it spreads out equally in all directions. If the source can be regarded as a point, the disturbance will, at a given distance from the source in any direction, be in the same phase. This is true of the electro-magnetic waves of light. These waves do not travel by means of the molecules of a substance, but they are influenced by these, and hence their velocity varies in different media. The velocity of all wave lengths is the same in free space (and practically so in air) but this is not the case when the waves are travelling through a medium.

It is just this difference in the velocity of the different wave lengths in a medium which is the cause of the familiar phenomenon of refraction. Since a disturbance spreads equally in all directions in a homogeneous medium, therefore at all points on the surface of any sphere with the source of the disturbance as centre the phase of the wave will be the same, and at a distance from the source a small area of the spherical surface may be considered as a plane surface. Let AB, Fig. 7, represent such an area (the plane of the area being at right angles to the plane of the paper), the direction in which the wave is travelling being aA perpendicular to AB. SS′ represents the surface of separation of two media, M_1 and M_2, in which the light travels with velocities V_1 and V_2 respectively. The point A of the wave front will reach the surface of the second medium before the point B, and entering it will travel to A′ by the time B reaches the surface at B′ and the wave front will then be A′B′. If V_2 is less than V_1 it is clear that AA′ will be less than BB′, and A′B′, instead of being parallel to AB, will be inclined at an angle to it—that is to say, the direction of propagation of the wave will be changed from aA to A′a′ where A′a′ is at right angles to A′B′, or the wave in entering the second medium has been refracted. It is the ratio of the velocities in the two media which is known as the refractive index μ, i. e.

$$\frac{\mathrm{V}_1}{\mathrm{V}_2} = \mu.$$

In any one medium the longer the wave length the greater the velocity—that is, the greater would be the distance AA′, Fig. 7—and therefore the less change there would be in the direction of the wave on entering the second medium. This is often expressed by saying that the longer the wave length the less it is refracted. If the incident ray is normal to the surface there will be a change in velocity but no change in direction on entering the second medium. Electro-magnetic waves may exist with wave lengths which vary from miles to 10^{-8} cm. or less. It is only a very small portion of these which, when they fall upon the retina of the eye, cause the sensation of light. Others may be detected by their heating effect, and others again by their chemical action and so on. All these different effects, however, are due to differences in wave length, and all the waves which cause

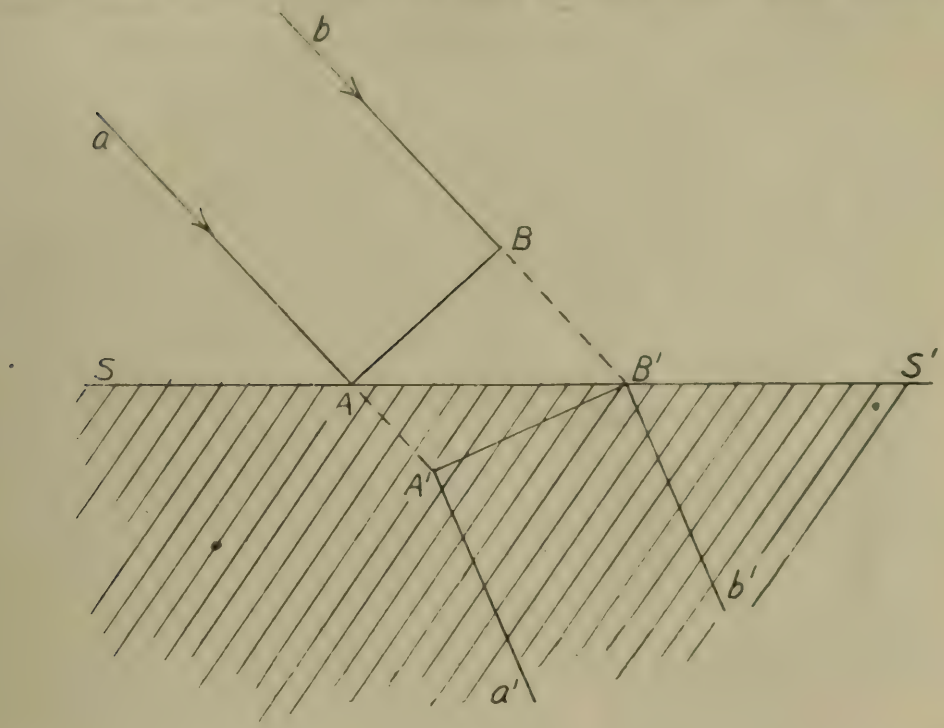


Fig. 7.

them are refracted when passing from one medium to another and travel in the same medium with velocities depending on their wave lengths.

It is this refraction due to the difference of velocity which is the cause of white light being split up into coloured components when passed through a prism. This will be made clearer by comparing Fig. 8 with Fig. 7, with which it is lettered to correspond, the suffixes R and V indicating the difference between the red and violet rays, the red waves being the longer and therefore least refracted. On emerging into the air the process described above is reversed. The red and violet rays form the limits to the waves to which the eye is sensitive, and the coloured band lying between them is known as the visible spectrum. The rays which are less refracted than the red and more refracted than the violet, and which therefore lie on either side of the visible spectrum, are termed the infra-red and the ultra-violet rays respectively. By means of an instrument, the spectrometer, based

upon the above principles, the wave length, and therefore the frequency of a wave corresponding to any point in the spectrum, may be accurately determined.

White light is made up of all wave lengths and is thus comparable to an utterly discordant chaos of sound of every audible pitch, and so without tone or colour—colour sensations being due to the stimulation of the eye by particular differences of frequency. Some sources of light, however, give out waves of only certain frequencies, and in this case, as would be expected, most of the spectrum is dark, with only a few bands or lines in it, e. g. the two D lines due to a sodium source. It has been found that in this way every known element has its characteristic spectrum, the lines composing it lying both in the visible and invisible part. It is owing to these characteristic line spectra that certain of the elements have been discovered, e. g. helium was first found by spectrum analysis to be present in the

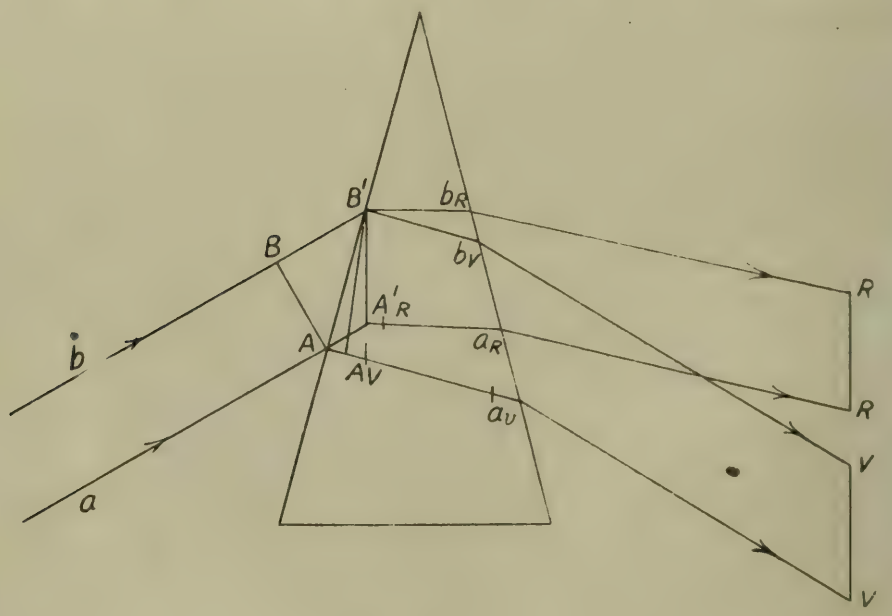


Fig. 8.

sun. By this means more than thirty-six elements present on earth have been identified in the sun.

X-rays. Of great importance to the electronic theory is the work that has been done on X-rays. W. H. Bragg and W. L. Bragg have shown that X-rays differ from light only in having a shorter wave length—the wave length of light being of the order of 10^{-5} cm. and that of X-rays 10^{-8} cm. X-rays are produced when the cathode rays (electrons), due to an electrical discharge in a vacuum tube, impinge upon any substance. They are usually obtained by arranging the tube so that the cathode rays fall upon a specially placed piece of metal, the anticathode.

It was demonstrated by Barkla that substances emitted characteristic X-rays under suitable stimuli. Barkla compared the X-radiations by their absorption in aluminium and found that each element gave at least two characteristic radiations, which for the elements examined fell into two series which he called the *K* and *L* series. Bragg, by using the face of a crystal as a diffraction grating, was able to

measure the wave-length of the X-radiation falling upon the crystal, and in this way to show that each element gave a characteristic spectrum with 'lines' or 'peaks' corresponding to definite wave lengths just as in ordinary spectra. He was also able to show conclusively that these peaks were caused by the rays of Barkla's *K* and *L* series.

A remarkable discovery was made by Moseley, who was lost to science in the Gallipoli campaign in 1915. He undertook the examination of all the known elements which could be brought under examination and found that the square root of the frequency of the X-radiation was proportional to the atomic number of the element for both the *K* and *L* series. The atomic number is the number indicating the order of the element in the Periodic Table, and is about half the value of the atomic weight. This is to say that the X-ray spectrum of any element is obtained by adding the same quantity to that of the element before it in the series. Since the atomic number is supposed to be the number of free positive charges contained in the atom, and therefore the number of free electrons, this is evidence that X-radiations at any rate depend upon the electrons of the atom, and that each element differs from the one before it by a single positive charge.

The photo-electric effect. It has been pointed out that X-rays possess the power of ejecting electrons from substances and that they differ from light only in their wave length, the question therefore suggests itself whether this property belongs to all electro-magnetic wave lengths or only to very short ones. Experiment shows that when light falls upon a metal the metal becomes positively charged, this charge being due to negatively charged electrons being emitted. It is found, however, that for each metal there is a certain wave length, and that wave lengths shorter than this wave length will cause the emission of electrons, but that wave lengths longer will not. This phenomenon of the emission of electrons by light is called the 'photo-electric effect', and the liberated electrons 'photo-electrons'. There is evidence to show that the electrons ejected are the electrons of the atoms, and not the free electrons present in a metal which take part in electrical and thermal conduction.

Since the attraction of the electron to the nucleus must be great, it would appear that a large amount of work must be done in removing it from the atom, and it is at present very difficult to see how sufficient energy can be obtained from the light wave for this purpose. An explanation given is that the frequency of the light wave must be such that it can act upon electrons vibrating in the atom if they happen to possess certain frequencies and in some way free a store of energy. Whether the energy is stored in the electron or the light is however at present an unsolved problem.[1] In addition to the energy required to free the electron from the atom, it must possess when freed sufficient energy to emerge from the metal. It has been suggested that all wave lengths may be able to free the electron from the atom, but that only the shorter ones can supply the additional energy to enable it to come out of the metal. It is found that the velocity with which the ejected electron emerges varies, and is proportional to the frequency of the liberating wave length, but it is independent of the intensity of the light.

[1] See page 44.

The majority of metals do not show the photo-electric effect for visible light radiation, but only for rays which lie in the ultra violet.

Phosphorescence and fluorescence. A photo-electric effect accompanies phosphorescence, but not as a rule fluorescence. It is suggested that in the latter case the light causes the electron, not to be expelled from the atom as in the photo-electric effect, but to be displaced within the sphere of influence of the molecule, and that the fluorescent light is due to it again settling down to its equilibrium position.

Albumin, gelatine, ivory, glycerine, and paraffin, when frozen solid by liquid air, fluoresce in the beam of the arc light; they absorb ultra-violet rays and transform them into visible fluorescence.

Photo-electric cells have been constructed whereby light energy is transformed into electrical energy. The general construction of a photo-electric cell is shown in Fig. 9. A is a fine platinum wire secured in the paraffined cork in the base of the tube, drawn tight against the inner surface, and fixed over the top edge by soldering on to stouter copper wire round the outside of the top of the tube. A thick platinum wire, B, is placed at the back to act as the second electrode. A is thus made to enclose a thin film of the liquid with which the cell is filled between itself and the wall of the tube. Any change in the composition of this external film will not diffuse rapidly into the rest of the cell liquid, and differences of electrical potential produced will be detected by means of the galvanometer. A saturated solution of chlorine peroxide, or a 20 per cent. solution of uranium nitrate in methyl alcohol can be used in the cell. The action of light on these cells, just as on a photographic plate, is prevented by low temperature (Dewar).

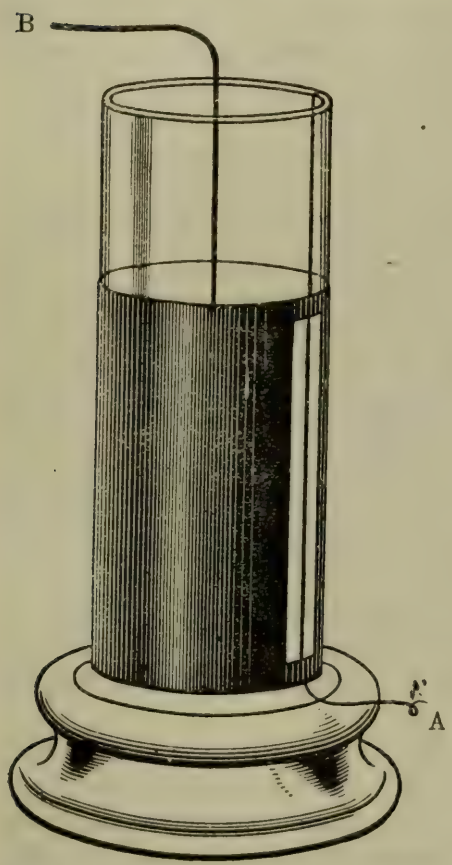


Fig. 9. Photo-electric cell. Dewar.

Physicists have used the photo-electric effect for energy measurements in radiometry, and in doing so have assumed the linear relation between the photo-electric current and the light intensity. The photo-electric cell constructed for this purpose is said to be as convenient to handle as a thermopile and a sensitive galvanometer, and moreover enables one to deal with light intensities of a far smaller order of magnitude. Its sensitiveness is comparable with that of the human eye (Hughes).

In the case of the photo-electric effect a positively charged layer on the surface of a metal assists, and a negatively charged layer retards, the emission of electrons by light. While the alkali metals are particularly sensitive, their halogen salts are quite insensitive to

light, but after bombardment with cathode rays these become extremely sensitive, owing, it is said, to free alkali metal. The sensitiveness of each alkali metal reaches a maximum at a particular wave length; the maxima shift towards the red on passing from *Li* to *Cs* (Hughes). The region in which the selective effect appears is one of high reflecting power. These facts are of interest with reference to colour vision, and the possible existence of substances in the retina sensitive to different wave lengths of light. Cells containing colloidal alkali metals are extraordinarily sensitive to light, e.g. a measurable current was obtained by Kemp when the cell was illuminated by a candle at 2·7 miles distance, a sensitivity of the same order as the retina. It is suggested that the increased sensitiveness of the colloidal modification is due to the greater ease with which electrons can emerge from the tiny globules of metal which characterize the colloid state—the average diameter of the globules is probably less than 10^{-5} cm. ($\frac{1}{10}\,\mu$). The retina gives an electrical response both to light and dark, and Gotch showed that the response to red, green, or violet, while of the same form in each case, differed in latent period and magnitude.

Joly [1] put forward the theory that the emission of electrons, probably by the pigment layer, is responsible for light sensation, and that when these electrons act upon the cones they excite colour vision. In the photo-electric expulsion of electrons by light, the range of the electron increases with the frequency. Hence for violet light the cone would experience a different distribution of the stimulus from that for red light, and so on. Lodge [1] suggests that 'the stems of the retinal substance act as accumulators, storing up an ether disturbance of quite unphysiological frequency until a quantum [2] has been collected, when a stimulating projectile is liberated'. The photographic image may be similarly initiated by electrons.

The sensitiveness of a metal surface to light decreases with time —'photo-electric fatigue'—and just as 'passivity' of metals is attributed to the formation of an imperceptible surface layer of oxide, so is this 'fatigue' associated with gases or vapours condensed on the surface. Similarly fatigue in muscle is attributed to H ions concentrated on the membrane of the fibres.

The application of the electronic theory to physiology has been exemplified in the preceding speculation. The following are further illustrations.

A substance can be brought into colloidal solution provided the particles are made so small that Brownian movements keep them suspended and agglomeration is prevented by a suitable surface film. So long as the particles are all charged negatively or positively they repel each other. Neutralization of the charge causes agglomeration. Uncharged suspensions are produced by adsorption of substances in solution with a suitable change of surface tension.[3]

The living cell appears under the microscope, using dark ground illumination, to be a colloidal solution containing an immense number of minute particles; the larger ones are the less stable. The particles

[1] *Nature*, Jan. 1, 1920.
[2] See page 44.
[3] W. D. Bancroft, *J. Phys. Chem.*, 1914, **18**, 550.

show Brownian movement due to bombardment by molecular vibration. They are regarded as aggregates or condensations of molecules, albumin, lecithin, &c., many being attached to ions of electrolytes present in the liquid, which give their surface a charge; a complex, such as protoplasm, of liquid, solid, and sometimes gas, minutely subdivided and intimately mixed, affords an enormous surface of interfaces in which atomic energy has play. The specificity of atomic energy of each of the many elements present in protoplasm allows an infinite number of groupings in the molecular aggregates which are held in a state of dispersion and mobile equilibrium by the play of energy within and without the cell.

Cellular structure results from reactions at interfaces producing membranes,[1] &c. The growth of structure is the result of energy received from the environment acting on an ovum attuned to that environment. The living matter varies in structure, forming countless species in accordance with the energy received in the countless variations of environment.

The remarkable combination of properties of water play a great part in living processes. These are fully considered by Bayliss.[2]

We may mention here:

The high dielectric constant (or specific inductive capacity). According to the electron theory this is greater the larger the number of electrons in a given space of a substance.

The higher the dielectric constant of a solvent the greater the dissociation of the ions of a solute.

The higher the dielectric constant the greater the number of electrons present in the substance, and the nearer the ions can approach to each other without recombining as molecules.

The fact that water does not decompose the salts dissolved in it but, by causing their dissociation into ions, enables all kinds of reactions to take place which do not occur between solutes in the molecular state.

Free ions are present in all watery solutions of electrolytes whether a current passes or not, a univalent ion carrying one free electron and a bivalent two, and so on. In the dissociation of electrolytes the charged particle, the hydrogen ion for example in the case of HCl, is very different from a neutral atom of hydrogen. Owing to the different rate of migration of ions, when a solution of an electrolyte is in contact with water, a potential difference is nearly always found at the boundary surface. While the two ions have an unequal rate of diffusion they cannot separate far owing to electrostatic attraction. Ions are greatly retarded owing to their carrying water molecules with them. The affinity of the 'ionic nucleus' for water mainly occasions the energy required for dissociation into ions; the hydration of ions is accompanied by evolution of heat.

The surface of contact of a liquid with a solid, gas, or another liquid, which does not mix, has the properties of a stretched film, due to the attraction between the molecules of the liquid. The contact surface is nearly always the seat of an electrical charge. This charge can be

[1] Substances accumulate at the surface which reduce the surface energy of the protoplasm; thus a viscous fluid may finally become a rigid gel. Mechanical stresses operate on the semi-rigid substance of the cell-wall.

[2] Bayliss, *Principles of General Physiology*, London, 1918, and *Nature*, Sept. 25, 1919.

increased, lessened, annulled, or reversed in sign by the addition of electrolytes to the liquid.

In any charged body, as Faraday showed, the charge is accumulated at the surface. The effect of an electrical charge on the surface is to reduce the surface tension from the repulsion of the particles of the surface which are all of the same sign. The area of the charged surface tends therefore to increase.

Now as any reaction that diminishes free energy always tends to take place, if a surface has a negative charge positively charged particles will concentrate upon it so as to annul its charge. These particles may be positive ions (cat-ions) or charged aggregates, e. g. colloidal particles. A substance in solution may then become of higher concentration in a surface film if it has power to lower surface energy ; thus the phenomenon termed imbibition or adsorption is produced.

As surface reactions depend not on the mass of reagent but on the extent of the surface, changes in the state of aggregation of colloids by which their surfaces can increase or diminish a million-fold are a potent influence, for the capacity factor can alter very greatly within a very small space.

The cell membrane takes a very important part in living structures. There is evidence that certain electrolytes act mainly on the membranes and do not penetrate, e. g. potassium on muscle, alkali on sea-urchin eggs.

Their relative impermeability makes cells bad conductors, but as this diminishes when cells are excited to activity, resistance to passage of a current then diminishes. The high electrical resistance of red blood corpuscles is due to the impermeability of their surface to ions, an impermeability broken down by haemolysins. Bayliss says 'the ultimate source of differences of electrical potential in cells must be due to the separation of electrically charged ions. The cell membrane permeable to one only of the opposite charged ions of a binary electrolyte is capable of accounting satisfactorily for all the phenomena, or a contact surface between two phases, one ion being soluble in both, the other in one phase only.'

The electrical change which accompanies the photo-synthetic action of the green leaf, the current produced in muscle on contraction, in nerve accompanying transmission of a nervous impulse, in a gland on secretion, in the retina on exposure to light and dark, demonstrates that electrons are set drifting in all vital reactions. In the electric organ of the fish, while the production of heat is minute, a potential of 450 volts may be reached. Probably, says Bayliss, 'the chief process is one of change of permeability involving redistribution of ions'.

In the case of muscle it is suggested that liberation of H ions is provoked by the stimulus, and that the contraction is due to the action of these charged particles on the colloidal system of the fibre, a surface tension phenomenon. The H ions rapidly diffuse and are rebuilt with the aid of oxygen into the muscle substance after relaxation (Hopkins and Fletcher, Mines). For the production of heat in muscle which takes place not only during the period of contraction but in the subsequent period of relaxation, oxygen is not required (A. V. Hill).

The respiratory centre is susceptible to the slightest alteration of H ions in the blood.

All forms of living matter produce H ions, and the accumulation of acids is one of the main causes of the cessation of growth and activity. Proteins, amino-acids, and certain salts such as the soluble phosphates and carbonates act as 'buffers', linking the hydrogen ions and forming neutral particles. The proteins and amino-acids similarly take up hydroxyl ions. The neutral reaction depends on the ratio of the concentration of the two ions. The living substance must be held in the lightest balance, there must not be a concentration of any substance so as to lock this up in firm union and prevent the ceaseless flux of reaction (B. Moore).

A very slight increase in the hydrogen ion concentration inhibits the growth of anaerobic organisms. Thus the growth of those organisms in bread which produce 'Rope' is inhibited by a slight degree of acidity such as yeast can tolerate; a little more and yeast also is killed. Similarly a slight increase in the hydroxyl ion concentration quickly reduces the rate, force, and output of the heart-beat, and eventually stops it. For example 0·02 to 0·03 normal solution of sodium bicarbonate added to Ringer-Locke fluid has this effect, which can be obviated by breathing through the solution and so adding CO_2.[1]

Oxygen becomes 'active' when changing from a bivalent to a quadrivalent element that is in the process of alteration of electric charge. There is for example no reaction between dry oxygen and phosphorus, but in the presence of water hydrogen peroxide is formed and phosphorus oxidized.

Catalysts, either tissue oxidases or artificial (e.g. an association of colloidal hydroxide of iron or manganese with an emulsoid colloid) set free 'active' oxygen from hydrogen peroxide and this oxidizes stable substances such as glucose, an emulsoid colloid maintains the 'active' constituent in a high state of dispersion and protects it from aggregation by electrolytes. Catalysts may be traces of 'active' material so maintained.

Pure oxygen and hydrogen even in the presence of water vapour will not explode when heated above the temperature which causes explosion to take place when impure. There must be an electrolyte present to start the reaction.

According to Ostwald the process of reduction is one of diminution of charge.

Moore points out that not only in oxidation, but in every biochemical reaction of zymolysis, fermentation, immunity, there is a triad of constituents to the reaction. One of these is 'active', and one links on the 'active' substance to the third which undergoes change. The key, the hand that puts the key into the lock, and the lock (Ehrlich). For example, in haemolysis, (1) the complement, (2) the serum sensitized to these, (3) the red corpuscles; in bacteriolysis, (1) the complement, (2) the amboceptor, (3) the cell; in digestion, (1) the ferment, (2) the co-ferment acid, alkali, or neutral salt, (3) the substance to be digested; again, (1) the adsorpate, e.g. a dye, (2) the mordant such as a salt of iron, (3) the adsorbing tissue.

Imponderable traces of substances thus produce the most powerful and specific biological effects. Traces of zinc (1 part in 25 million) added to the culture fluid used for growth of a mould—aspergillus—

[1] B. Moore, *J. Physiol.*, 1919. **53**, *Proc. Physiol. Soc.*, p. xxxvi.

increase the crop 50 per cent. So, too, with iron or manganese, but while vigorous growth of mycelium is obtained with iron or zinc alone no conidia form in the absence of manganese. Tetanus and gas gangrene bacilli washed clean and injected are innocuous. The injection of a few milligrams of an ionizable calcium salt at the same or a different site, at the same time or even three months later, secures infection.[1] The calcium ion is specific in breaking down the tissue cell defence.

One part of copper in 77 million of distilled water is toxic to spirogyra in one minute. Copper in distilled water likewise kills tadpoles and tubifex. Aspergillus cannot be cultured in a silver vessel. Such bright metals pass into the colloidal state in pure distilled water and become active.[2]

The influence of traces of metals on the *Photobacterium phosphorescens* is shown by placing a piece in the middle of the dish containing the infected medium and later photographing the phosphorescence (Fig. 10, p. 51). Gold, the platinum group, tantalum, selenium, cadmium, magnesium, and tin have no effect. Sulphur stimulates; bismuth, thalium, lead, nickel slightly check growth; iron, aluminium, zinc, copper have a stronger checking effect; the more deadly metals are cobalt, silver, mercury, antimony, arsenic.[3]

Spermatozoa are negatively charged and move only so long as this charge is maintained by a certain concentration of hydroxyl ions in the medium.[4]

Artificial fertilization of sea-urchin eggs is obtained by the addition of certain ions to sea water, in the frog by pricking the membrane of an egg with a glass needle, a fatherless frog being thus produced.

The explanation of specific action in immunity, anaphylaxis, &c. is to be looked for in a colloidal precipitation process at the surface of the sensitive cells by which their semi-permeability is more or less destroyed (Bayliss).

Anaphylactic shock can be prevented by rendering the blood hypertonic with sodium chloride before injecting the specific serum, or by adding hypertonic salt to the serum. So, too, with haemolysins. The linkage of the active poisonous substance with the cells is thereby prevented. The common cause of all these phenomena is the disturbance of the delicate equilibrium between the colloids of the blood, tissue cells, and electrolytes, united or adsorbed in common solution or suspension,[5] a union which regulates the state of aggregation, the osmotic pressure and water-swelling, and the diffusive power of the particles of a suspension of lipoidal nature such as exists in the cells.

It has recently been shown by Zwaardemaker[6] that the place of potassium (a radio-active element[7]) in Ringer's solution can be taken by radiation from radium, so far as regards the maintenance of the frog's heart-beat, prevention of oedema, &c., a direct proof of the action of atomic energy in the phenomena of life. Radio-active substances

[1] Gye (Bullock) and Cramer, *Proc. Roy. Soc.*, 1919, **B. 90**, 513.
[2] Bayliss, *loc. cit.*, 222.
[3] Dewar, *Roy. Instit. Lect.*, Jan. 10, 1910.
[4] J. Gray, *Proc. Roy. Soc.*, 1920, **B. 91**, 147.
[5] B. Moore, *Brit. Med. Journ.*, Oct. 18, 1919.
[6] *J. Physiol.*, 1920, **53**, 273.
[7] N. R. Campbell and A. Wood, *Proc. Camb. Phil. Soc.*, 1906, **14**, 5.

emitting either β- or α-rays are effectual, but carefully counterbalanced neutralize each other. Fluorescin sensitizes the heart to radio-action.[1]

The relation between reflection, absorption, and transmission explained by the electronic theory. When radiation falls upon a substance it may be either reflected, absorbed, or transmitted. If reflected, it obeys the laws which are stated, by saying that,—the incident and reflected ray are in the same plane and make equal angles with the normal to the surface at the point of reflection. The portion of the ray which is not reflected enters the substance and suffers refraction as already described, and it is this portion of the ray which is either absorbed by the medium in which it is travelling, or is transmitted, and emerges at the second surface of the medium. It is the absorbed portion of the ray which causes any action in the medium, e. g. heating effect, chemical action, &c.

Different substances show the above characteristics to various extents, for example, glass transmits most of the visible radiation, but not the longer infra-red heat rays, water absorbs heat rays, while metals absorb the visible rays. It would therefore appear that the atoms of the material must be the determining factor, but since no substance treats all waves alike we are led to the conclusion that it must be the frequency of the incident radiation in connexion with the atoms of the substance which decides what happens to the ray.

The rapidly alternating electric fields which constitute light may be supposed to set the electrons in a dielectric (non-conductor) into forced vibrations of its own period. These secondary vibrations are emitted in all directions, and interfere with each other, and with the primary vibrations which form the incident light. The result of this interference outside the dielectric is the propagation of a reflected wave, inside of a refracted wave.

When a conductor is placed in an electric field, the induced electrification on its surface is such as to neutralize the electric intensity in its substance. Accordingly, when such a body is placed in the alternating electric field which constitutes light, the disturbance cannot penetrate into the body, but must be either reflected or absorbed. Absorption occurs if the motion of the electrons is hindered by frictional forces. Metals and all good conductors of electricity are then opaque.

The absorption of light is determined, like the resistance to a steady current, by the rate at which the energy of the electrons, acquired within the action of the field, is dissipated by collisions.

When the molecules in which the vibrating electrons are contained collide with other molecules, the energy of the vibrating electron is transformed into translatory motion of the molecules, producing a rise in the temperature of the substance.

Since the reflected, absorbed, or transmitted parts of a ray must when added together be equal to the original ray, it follows that the greater one part, the smaller must be the others, for example, a ray which is totally absorbed can be neither reflected or transmitted.

When a body is first heated it sends out waves of low frequency (or long wave length, refracted least by a prism). As the heating is

[1] It is the charge of the α- or β-particle which is the effectual agent; the mass of an α- particle is at most $\frac{1}{5000}$th of that of a molecule of albumin. The millionth of a milligramme of radium per litre gives a physiological effect. There are 40 grammes of potassium in the body; this amount exerts a very feeble radio-action; distributed the action must be inconceivably small.

continued, waves of higher frequency are added, until at 500° C. the waves are quick enough to excite the sensation of red light. The waves exciting violet are added at about 1400° C., the other spectral colours between. Further heating enables the body to send out waves of very high frequency which produce chemical and electrical effects and are lethal to protoplasm. All the waves travel with the same velocity in a vacuum and only when absorbed produce heat or chemical effects. 'For every temperature of a heated substance there is one particular wave length of which more radiant energy is emitted than of any other. In passing to the temperature of a white heat, not only are waves of a shorter and shorter light emitted as the temperature rises, but also the radiation of the longer waves is enormously increased at the same time, so that white-hot bodies give out far more energy as dark heat rays than as visible light rays. The maximum energy shifts further along the infra-red regions towards the visible region as the temperature of a glowing substance rises.'

This is expressed by Wiens' law, which states that if a body is emitting full radiation then the wave length of maximum radiation λ_m is given by $$\lambda_m \theta = \text{constant},$$

where θ is the absolute temperature of the surface.

A vibrating body absorbs vibrations similar to those which it emits, and a body which absorbs any particular kind of radiation will in general, when heated, become a source of radiation of the same kind. A tuning-fork or stretched string vibrates in unison with another of the same pitch. Suppose a screen of strings were set up tuned to the note *a*, if a mixed sound were produced beyond the screen the sound *a* would not be heard. So atoms take up the frequencies to which they are tuned, and appear opaque to these, e. g. if white light passes through sodium vapour, waves with the same period as the sodium molecule are absorbed. The core of the sun at white heat gives out a continuous spectrum, but the radiations corresponding to the periods of the atoms in the sun's atmosphere are absorbed—hence the Frauenhofer dark lines seen in the solar spectrum.

In the case of an ordinary opaque body the greater part of the energy of wave motion is absorbed and transformed into heat; in the case of the eye into chemical or photo-electric effects which transformed into nerve impulses arouse sensations of light in the brain; in the case of a photographic plate, or a green leaf, into energy which produces chemical decomposition.

Hence the terms heat rays, light, and chemical or actinic rays are used, but it must be understood that the same series produces different effects according to the absorbing power of the bodies for the different frequencies. All these waves can be absorbed by a black body and produce heat, but the long waves of small frequency used for wireless telegraphy are not easily absorbed and pass through solids.

Action of solar rays on life. Considering the evolution of living matter Moore points out that the short wave-lengths acting on iron in colloidal solution in the presence of carbon dioxide build up formaldehyde; further, they can bring about a condensation of formaldehyde, and thus the formation of reducing substances leading to carbohydrates. Further, there is a general reversible action as a result of which

formaldehyde, a poisonous substance, is produced in all intense reactions of light upon substance of bio-chemical origin. 'The energy of light which is essential to healthy growth and the upbuilding of organic material from inorganic, supplied in a wrong fashion and not shielded in its onset, may reverse these delicate processes and cause death and degeneration of living substance.' [1] Thus light is the most universal and potent natural germicide and purifier.

The pigments of those micro-organisms and fungi which can flourish in light always absorb the blue rays. Chlorophyll screens the plant protoplasm of the leaf, and secures by selective absorption the conversion of sunlight into chemical energy. Energized by sunlight plant protoplasm grows, assimilating carbon dioxide and oxides of nitrogen from the atmospheres and crystalloids dissolved in water. In the spring the sun gaining a higher altitude stimulates the growth not only of green plants on the earth, but of the vegetable plankton of the sea, which support all animal life.

In the evolution of protoplasm there naturally became developed a surface layer exposed to and energized by sunlight, and a deeper sheltered layer which imbibed the chemical energy stored by the former, becoming thus to this extent parasitic, while reacting to the watery habitat, and the dissolved electrolytes and neutral molecules therein. From this parasitic tendency animal protoplasm may have become developed with all the manifold mechanisms for seeking and securing food. As protoplasm directly exposed to sunlight is destroyed by it, animals are screened both by living in the shade and by impenetrable surface layers, but sensitivity to sunlight has been perfected in visual sense organs as a means of securing a beneficent absorption of warmth, of avoiding injury, securing food, seeking a mate. All the energy transformed by the animal is finally degraded into the universal sea of heat, the atomic energy of the food-stuffs, so far as we can see, not being set free but returned to the world in the waste material excreted by the animal, to be once more built up by the sun into plant bioplasm. The living world is then kept going by radiant energy, and the environment of the human body in relation to the receipt of radiant energy and the escape of heat, the final degraded form of energy, is of not less importance than it is in relation to the food supply.

The sunlight pulsing in at the eye and transformed in the retina into nerve energy excites conscious vision and memory with associated tones of feeling, and influences powerfully the whole bodily activity, which are played upon, also through other avenues of sense, by smell and taste, most effective in arousing emotions, by the impulses of sound acting upon the cochlea, by light and heat rays upon the skin, kinetic energy of molecular matter acting upon skin, joint, and muscles, and semi-circular canals, and also through the blood by the chemical energy of the food and oxygen.

On the peripheral parts of the retina, the threshold stimulation of light (not colour) is limited to between the orange and blue—wave lengths 600 and 440 $\mu\mu$ of the spectrum.[2] The curves expressing the

[1] B. Moore and T. A. Webster, *Proc. Roy. Soc.*, 1918, **B. 90**, 168.

[2] The maximum for the light adapted eye is 590 $\mu\mu$ in the yellow, for the dark adapted 505 in the green. Cf. H. Weve, *Arch. néerl. de Physiol.*, 1920, **4**, 243.

threshold exciting energy, the bleaching effect on the visual purple, and the amount of light absorbed by it, all follow the same course, a powerful argument in favour of the participation of the pigment in vision.[1]

The dark heat waves have a frequency less than 390 billion per sec. This frequency is too small to affect the retina, or, in ordinary cases, to agitate molecules so as to shake them to pieces and operate chemical decomposition, but Abney has secured photographs by these waves, employing as a sensitive material molecules which are specially heavy and complex.

The invisible ultra-violet rays with frequency above 760 billion per sec. produce no effect on the retina; they are absorbed by the cornea. These rays are very active in causing chemical decomposition. The light rays, the frequency of which lie between 390 and 760 billion per sec., also have heating and chemical effects. Thus the yellow and red rays are the most active in bringing about the decomposition of carbonic acid by the green leaf.

The ultra-violet and visible rays which reach the earth from the sun have an aggregate energy as measured by the thermopile far less than that of the longer red or dark heat waves.

Energy curves of sun and other light. According to the measurements of Langley, the energy of the sun spectrum is distributed thus :—

Ultra-violet region	1/100 th's
Visible „	19/100 „
Thermal beyond red	80/100 „

While it is in the red, yellow, and green region of the solar spectrum that the maximal evidence of chemical energy is found, the arc light is rich in short chemically active rays which have not been absorbed by the atmosphere as in the case of sunlight.

The quantity of chemical decomposition is within wide ranges of intensity the same when the product (time of exposure and intensity of light) is the same—the basis of calculation of 'exposure' in photography.[2]

In the case of radiation just considered, the light is emitted as a result of the body being heated. There is another kind of light—luminescence—in which electric energy or chemical energy is changed directly into the energy of light waves without passing through the intermediate stage of heat. All flames, the carbon arc and metal filament lamps, the incandescent mantle, are cases of temperature radiation. In the case of the metal filament lamps, and particularly the incandescent mantle, an unusually large proportion of the radiation falls within the visible spectrum owing to the attainment of high temperatures, but as the temperature is increased this proportion tends to become less, so that not much further progress in illumination is to be expected on these lines. A vacuum tube through which induction currents are passed gives luminescence, the temperature within the tube being only 30–40° C. The light produced by living organisms—glow-worms, fire-flies, phosphorescence of sea organisms, deep-sea fishes—is luminescent, and produced apparently

[1] Bayliss, *loc. cit.*, 522. The same group of vibrations give rise to light reflexes in all classes of animals (Carl Hess).

[2] This holds good for the bending of seedlings towards light (Blaauw).

without expenditure of energy as heat, the luminous efficiency being 100 per cent.

When the rays are allowed to fall on the thinnest black paper no heat can be detected on the opposite side of the paper (Barnard).

The spectrum is limited to a narrow band in green blue, a ghastly light, but one which could be rendered cheerful by a fluorescing red screen.

Molisch, also Barnard, made lamps out of flasks coated with salt-peptone-gelatine, and coated with phosphorescent bacteria. Barnard could recognize a face two yards away by the light of these, and read large-sized print. The lamps last 2–3 weeks.

The light is produced by an oxidation process, and the presence of water is necessary. A substance called photogenin is separable from the cells of the glow organ, and this can be activated to shine in the presence of oxygen by solutions of tissue extracts, sodium chloride, thymol, saponin, &c. A number of compounds are known which emit light[1], e.g. pyrogallol in weak solution oxidized by plant peroxidases, haemoglobin, or potassium permanganate in presence of hydrogen peroxide.

There are many substances from which a luminous mixture can be made.

Sulphide of Ca, Sr, or Br, with a trace of added metal Bi, Ni, or Pb, and a flux such as Na_2 SO, or Na_2 B_4O_7 all fused together make a very phosphorescent substance.

The mercury vapour quartz lamp gives no red, few orange and yellow, many ultra-violet and visible rays. The ghastly appearance of skin when illuminated by such a lamp is due to there being no red rays to be reflected.

The luminous efficiency of an oil lamp is 1/10 per cent. of the thermal energy of the oil burnt—a colossal waste. That of a gas-mantle is given as 1·5 per cent., of a tungsten filament electric glow lamp 10 per cent., of a 5,000 candle arc at 3,000° C. 25 per cent., of solar radiation 25 per cent.

As the luminous efficiency of the gas-filled tungsten metal filament lamp is about 10 per cent. of the electric power used to produce it, and as about 8 per cent. of the thermal energy of the coal is turned into electric power at the average power stations, only 10 per cent. of 8 per cent., say 1 per cent., of the thermal energy of the coal is secured as light, and the rest wasted as heat. The case of the gas mantle is much better; for coke and valuable by-products, ammonia, &c., are left over after the production of gas, and from these some 10 per cent. of the thermal energy of the coal, used to generate the gas, may be utilizable.

The illumination of an object in ordinary daylight is roughly 1,000 foot candles.

From 1–4 foot candles is sufficient for office desks, schoolrooms, &c.

The radiant energy received by the out-door worker in diffuse daylight and the indoor worker in poorly lit rooms is then in some such proportion as 250 to 1.

When the sun shines the difference is immensely greater. Indoor life cuts us off then from an immense amount of radiation.

[1] Cf. Harvey, *J. Biol. Chem.*, 1917, 31, 311.

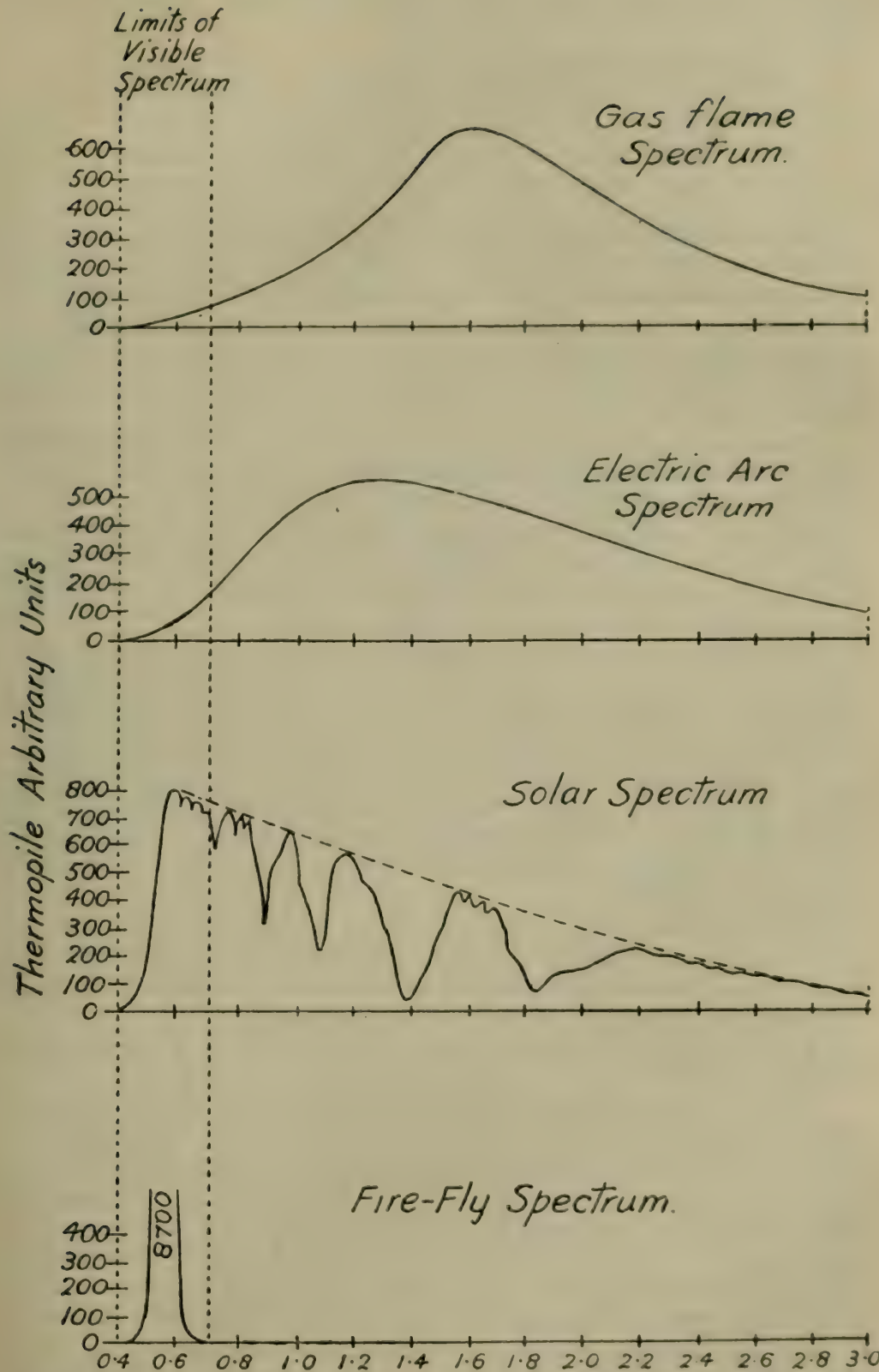


Fig. 11. Energy curves. Langley. The effective sun-rays lie between 0·3 μ and 2 μ.

Diffuse reflection of ordinary daylight in rooms depends on the nature of the walls and furniture. The ratio of light radiated to light received is for

White cartridge paper	0·80
Yellow wall paper	0·40
Emerald green paper	0·18
Black cloth	0·012
Black velvet	0·004

It is obvious then that gas and electricity can be saved by choosing a light wall paper and a light ceiling. So too with fire light, dark furniture and walls absorb the light rays and convert them into dark heat rays which warm the air. A room with white and polished surfaces reflect light rays too and fro, and the body is warmed by more radiant energy as such. The more the body is warmed by radiant energy and the less the air is heated the better.

The exchange of heat radiation between two bodies depends on their absolute temperatures, the nature of their surface, and their distance apart, the radiation from a body which reaches any point varying inversely as the square of the distance between the body and that point. Every body then emits heat radiation in all directions to an extent determined only by its temperature and the nature of its surface, and absorbs it from all sources to which it is exposed.

The absolute amount of radiation from a body does not depend on the condition or even on the presence of surrounding objects, but solely on the condition of the body itself, e.g. the absolute physical brightness of a candle is independent of the presence of illuminated objects.

Not only does a fire warm the room, but the walls warm the fire. The warming of a colder body by a hotter one depends upon the difference of two similar but unequally opposed actions. The body is cooled by an ice block placed near it because it loses to the ice more heat than it receives.

When one body, e.g. a thermometer, is surrounded by another, the body enclosed and the walls of the enclosure come to have the same temperature; this equalization of temperature by radiation is quite independent of the form of the walls of the cavity.

At constant temperatures bodies radiate as much energy as they receive.

It has been already stated that radiation falling upon a surface is reflected, transmitted, or absorbed by the substance. The reflecting power of any surface increases with the degree of polish of that surface. For diathermanous (transparent to heat rays) substances it increases with the angle of incidence, for metallic surfaces it is nearly constant up to about 70°. Polished metals are the best reflectors. The reflection varies with the nature of the source of radiant heat. If a material readily radiate heat away, it must, in order that the equilibrium of temperature be kept up between it and surrounding materials, absorb as much. Good absorbers are good radiators. Good reflectors or diffusers are bad absorbers. A brightly polished metal surface is a bad absorbent, as is shown by the circumstance that it is a good reflector; being a bad absorbent it is a bad radiator; a polished dish cover will retain heat much longer than a thinly blackened surface will. If soot be

sprinkled upon snow, the snow will more readily melt in the sun's rays, for the soot is a good absorbent and itself becomes heated. The back of the riding hand becomes glazed in the tropical sun ; it is made into a good reflector by the arrangement of the horny scales. The back of the hand exposed to sunshine is far more shiny than the fore-arm which is covered. Lampblack, reflecting and transmitting none, absorbs all rays ; other substances are selective, some rays being absorbed and others reflected. The diffusive power of a surface varies with the angle at which the rays from the source of heat strike the surface, with the nature and material of the surface—a fine powdered rough surface diffuses—and with the source of heat ; the less refrangible rays tend to be absorbed, the more refrangible to be diffused.

Rough lampblack absorbs 90 per cent., diffuses 10 per cent. of sunlight. A white lead surface absorbs 90 per cent. from a source at 100° C., and only 18 per cent. from solar radiation. Diathermancy varies with the nature of the material and the source of heat, for example :

Plate 2·5 cm. thick.	Oil flame.	Incandescent platinum wire heated in spirit lamp.	Blackened copper heated to 390°.	Blackened copper heated to 100°.
Rock salt	92%	92%	92%	92%
Mirror Glass	39	28	6	0
Quartz	37	28	6	0
Alum	9	2	0	0
Ice	6	0	0	0

A glass screen is heated more by a fire than the window pane is by the sun.

The coefficients for absorption and emission, determined by Leslie, Melloni, and others, for a given temperature of the body, and a fixed value of θ (excess temperature between body aud surrounding) have the same numerical value. For example, the emissive power of a surface of a Leslie cube filled with water at 100° C. was when covered with :

Lampblack	100 per cent.
White lead	100 „
Paper	98 „
Glass	90 „
Indian ink	85 „
Polished metals . . .	9·5–2·2 per cent.

The emission was measured by means of a thermopile suitably screened from any other source of heat.

Absorptive power was determined by using a thin copper plate on the front of which the substance to be tested was placed, the back radiating the heat absorbed to the thermopile.

Using a Leslie cube filled with water at 100° C. the absorptive powers were :

Lampblack	100 per cent.
White lead	100 „
Isinglass	91 „
Indian ink	85 „
Shellac	72 „
Metals	13 „

The emissivity of glass is very high for long waves. If that of

a 'black body' is 1·0, that of glass is over 0·96 at 2 micron wave length, rising to 0·99 at 8 micron, and falling to 0·72 between 9 and 10 micron, rising to over 0·9 again at 11 micron (cited after J. Harker).

The absorption and emissivity of glass for long waves is like that of clothes and the skin, and the Kata thermometer is comparable in this respect to these.

White or black skin, and clothes, are equally absorbent and emissive of dark heat.

The emissivities of metals are much smaller for long wave lengths (10 micron) than for visible waves.

The radiation in a uniform temperature enclosure requires notice. Suppose part of the surface of the enclosure is lampblack; this absorbs nearly all that falls on it, but makes up by an equally great radiation; part polished silver, this radiates very little, but reflects nearly all that falls on it; part rock salt, this neither reflects nor radiates much, but transmits freely most of the radiation from and to the surface against which it is placed. A body opaque to any kind of radiation emits that kind, and in a uniform temperature enclosure emission equals absorption.

If then we were placed inside an iron ball coated inside partly with lampblack, partly with silver, and partly with rock salt, and the ball were heated red hot, every part would appear the same uniform red glare and no boundaries would be seen, as is indeed the case if we put bits of glass within a cavern in a red-hot coal fire. In an enclosure at uniform temperature radiation is not changed by altering the materials of the walls.

A darkened room or cellar gives a near approach to a uniform temperature enclosure; the skin feels the uniformity. In a window-lit room radiations may be received from the sun at an enormously higher temperature than from the walls of the room, while to the clear winter sky at night radiations are given off far more than to the walls of the room.[1]

In considering the gain of radiant heat, the upper temperature is the one which is all important, the temperature of the receiving surface, provided the gradient is considerable, being of minor importance. Hence the superiority of sun or flame for warming the skin and clothes over the dark heat of steam or water pipes and walls of rooms slightly warmed by these or plenum system. The problem is how to secure direct and adequate exposure to radiant energy of each individual in rooms occupied by many, radiant energy which will allow an ample supply of cool moving dry air in place of a uniform stagnant warm moist atmosphere.

Ångström's pyrheliometer for measuring the radiant heat of the sun consists essentially of two metal strips blackened on one side and in every way similar. One of these strips is exposed to the radiation to be measured, while the other, which is screened by a double wall from this radiation, is heated by an electric current. The strength of the current is regulated so that the temperature of the two strips is the same, as read by a thermocouple and galvanometer. Then the energy expended by the current in the one strip is equal to the energy

[1] J. J. Thomson, *Heat* (Griffin, 1911), p. 239.

radiated into the other. The radiation of the sun in most favourable conditions is sufficient to warm the surface of the earth 2·1 calories per sq. cm. per min. The maximum radiant heat effect of the sun then is 33 millicalories per sq. cm. per sec. The effect on black clothes with high absorptive power may then be overpoweringly great.

The noon-day sun passes through a depth of one, the low sun through a depth of two atmospheres; the power of the latter is consequently decreased by absorption through water vapour. The lower atmosphere is comparatively transparent to ultra-violet light. The line λ 2536 Ångström units can be detected in the spectrum of a mercury lamp 4 miles distant. The solar spectrum is limited by atmospheric absorption at high altitudes to λ 2922. The limitation is due to scattering of light by ionized particles. The blue colour of the sky is caused by this scattering.[1] The blue rays are scattered much more than the red rays. The red sunset and sunrise are due to the rays having then traversed a greater distance of atmosphere and the blue rays being more diffracted and lost to vision.

While pure air is highly diathermanous—a spherical vacuum flask containing liquid air can be used as a burning glass—water vapour increases the absorptive power 30 to 70 per cent. In deserts where the days are very hot and the nights cold there is little vapour in the air. Vapour protects from excessive heat by day and cold by night. It moderates extremes. The moderated sun rays which reach the earth's surface through the humid atmosphere are absorbed by the earth, which is warmed and emits long heat waves; these cannot pass away through the aqueous vapour of the atmosphere. Were it not for the atmosphere the earth's temperature would be below −200° C. even under the vertical rays of a tropical sun.

Ventilation is set up by the sun heating and evaporating moisture from the ground; expanded by heat and diluted with water vapour the air is made lighter, rises, and is displaced by heavier cool air. Any kind of roof limits this ventilation while affording a shield from the sun.

On hot sunny days it was found impossible to use poison-gas clouds as an offensive. The vertical currents from the ground carried up the gas. In the early morning with a breeze the gas cloud rolls for miles.

Glass retains dark heat rays—behaving like a black body—thus the light that passes into a greenhouse, absorbed and converted into heat by the objects within, is trapped.

Glass is more or less transparent to all the radiation reaching us from the sun, while a glass plate 2 mm. thick absorbs half the radiation from an argand burner, and is as efficient a screen as a metal plate in front of a Leslie cube at 100° C.—hence glass fire-screens.

Clear mountain air is not heated by the sunshine; in the shade it may be very cold. If there be any particles of dust in the air, these being opaque to light will become heated, and the air is then, by conduction, rendered warmer. On clear cloudless nights the earth is cooled by radiation into the atmosphere, the temperature of which is low. The air in contact with the earth is chilled, and if its tempera-

[1] R. J. Strutt, *Proc. Roy. Soc.*, 1918, **A. 94**, 453.

ture falls below the dew point some of the water vapour in the air is deposited as dew. If the temperature falls below 0° the dew is deposited as hoar frost. Dew does not form under a tree or shelter, but only where there is free radiation. Wind hinders the formation of dew by continually bringing fresh portions of air into contact with the earth without allowing them to remain long enough to be cooled to the dew point. Dew is most copiously deposited on good radiators and bad conductors of heat such as grass, wood, wool, as these quickly cool on clear nights. Very little dew falls upon stone, slates, and metal surfaces, as they are good conductors. Much of the dew is transpired from the soil. The surface of clothes is cooled by radiation on a clear night and the humidity increased therein.

There is a marked difference between the radiation loss and feelings on cloudy and clear days and nights. The clouds absorb, reflect, and emit earth radiations and sun radiations. To the great loss of radiant heat is due the bracing feelings on cool, clear, frosty nights. Muggy, close feelings, on the other hand, arise from calm, cloudy conditions on account of diminished heat loss from radiation, in addition to the lowered heat loss from convection.

Air heated over steam pipes or furnaces has an unpleasant enervating quality—dead air. A hot body by giving out heat to colder air does work, causing the air to expand in opposition to pressure. The air is thus made thinner, and the partial pressure of oxygen less. The skin imparts the agitation of its particles to the particles of air, and these having a very small heat capacity, if confined, quickly assume a state of motion which imparts to the skin as much heat as it received. But if by convection a ceaseless stream of cold particles of air come momentarily in contact with the skin, and each is endowed with greater kinetic energy and then is swept on, the cooling power is very great. When air is heated over steam pipes, and delivered into a room as a means of heating the room, the temperature of the air particles is raised and approaches body temperature. The air is thus rendered 'dead', its heat capacity is greatly reduced, the particles striking the body cannot take up the heat from the skin, the molecules of the skin surface and of the air in contact with it are vibrating at the same rate. On the other hand, out of doors eddies of cold air are ceaselessly descending from the cold upper strata, and the air is never 'dead'.

In a small room crowded with occupants the radiant loss of heat from the body is very much less, owing to the warm surroundings.

Ionization of air. Good conductors of electricity are good conductors of heat, conduction of either electricity or heat being effected by the agency of free electrons just as conduction of heat in a gas is effected by the agency of molecules.

Free electrons at the hot end of a bar of metal have a greater kinetic energy than those at the cold end; collisions take place and so energy is transferred, i. e. heat conducted.

While free electrons move among the atoms of a metal as the molecules of gas move in a porous substance, few escape at ordinary temperatures because they are held at the surface by positively charged atoms. On heating, the kinetic energy of the free electrons rises and more escape. At temperatures over 1000° C. a current of negative

electricity (electrons) flows from all metallic conductors—the thermionic current.[1] Heated metallic surfaces may then ionize the air and increase the number of molecular aggregates of air and vapour which attach themselves to ions.

The phenomenon of ionization also takes place in ordinary flames such as a Bunsen burner. If two platinum wires connected with a battery are placed in the flame but not touching each other, a current will pass. The conductivity is enormously increased by placing a bead of a sodium salt in the flame below the wires. Using salts of various elements the conductivity is found to be in the order of the atomic weights.

The velocity of the negative ions is such as to indicate that they become loaded with neutral atoms.

Aitken[2] estimated that there may be as many particles in one cubic inch of the air of a room at night when the gas is burning as there are inhabitants in Great Britain.

Not only the gases of a flame but the gases liberated by the action of acids on metals possess considerable electrical conductivity for a short time after removal from the scene of action.

According to B. Moore oxides of nitrogen are formed in the air by the action of the short wave lengths of light and ultra-violet rays. South winds blow up air containing these oxides of nitrogen formed in the sunny south.

Impurities in the air, e. g. sulphur acids of coal smoke, increase the number of ions in the air produced by sunlight. These ions as well as dust particles act as nuclei for the condensation of vapour.

T. Aitken[3] found that when the wind was from the N.W. and the sky clouded the number of particles present in the air would be low, generally under 500 per c.c., but that if the sun kept shining all day the numbers rose to many thousands in the afternoon, after which the numbers decreased and again were very low next morning if the wind continued to blow from the N.W.

The rate of production of ions in the atmosphere is in part due to the radio-active matter (emanations from radium and thorium) suspended in the atmosphere, and to the penetrating rays from the radio-active material in the soil. The presence of radio-active emanations in the atmosphere is universal; they are found in fresh fallen rain and snow. These emanations arise from radio-active materials in the earth, and slowly diffuse away. A dry and porous soil and low barometric pressure favour the diffusion outwards of the air containing the emanations. As the radium content for sea-water is very small compared with the radium content of rocks, the active matter carried down to the sea by denudation must rapidly be deposited in the bed of the ocean. Caves and cellars, brick buildings, the soil, spring water, or sediment from spring water are richer than outside air in radio-active matter (McLennan). The fact that sea-water relatively has so little, while the seaside affords health resorts, is against the view that these emanations have any beneficial influence on health. The amount of radio-active emanation per cubic metre of air is expressed in terms of

[1] The magnetic deflection of this current is used to amplify sound in wireless telephony, &c., the thermionic valve.

[2] *Nature*, Mar. 1, 1888.

[3] *Trans. Roy. Soc. Edin.*, 1900, **39**, 15.

the weight of radium which would be in equilibrium with that quantity of emanation. The methods used for estimation are absorption by charcoal or condensing out with liquid air. It is estimated that 1 gram of radium would be required (per sq. kilometre of soil) to keep the amount found up for 10 kilometres height of atmosphere. The radio-active emanation is about equal all over the land and undergoes a diurnal variation, due to the radio-active matter in the atmosphere coming out of the soil or being deposited at different rates during the day.

Ions can be filtered out of air by passing it through cotton wool, or over plates charged at a sufficiently high potential difference.

The ionization of the air is measured by Ebert's apparatus. A constant current of air is drawn between two concentric cylinders by means of a fan actuated by a falling weight. The inner cylinder is insulated and connected with an electroscope. The capacity of the apparatus and the velocity of the current of air being known, the rate of movement of the aluminium leaves of the electroscope affords a definite measure of the number of ions present per c.c.

The value obtained when the inner cylinder is negatively charged is usually greater than the value when the cylinder is positively charged. The effect of mist is to increase the potential gradient but to so reduce the mobility of the ions that there is a substantial reduction of the air-earth current.[1]

As ions are removed from air by filtering through cotton wool, it has been suggested that filtration of outside air makes it 'dead'.

For example, air filtered through cotton wool and passed into the picture gallery at Buckingham Palace proved too 'dead' to be endured. While wet- and dry-bulb temperatures might be the same in this gallery as in the Palace at large, stagnation of air has the most potent effect on cooling and evaporative powers, and their reduction must be taken into account before we have recourse to the deionization of the air for an explanation of the deadness.

I have been informed that an air that blows over the mountains into Canterbury, New Zealand, has a very unpleasant quality, and that it was found to hold ions mostly of one sign. Here again we must know what the cooling and evaporative powers are, and the monotony of effect on the nerve endings, before we can justly ascribe the unpleasant quality to the ionization. People are far too prone to take refuge in the mysterious for an explanation when plain and potent causes are overlooked. Air sniffed from a vessel of water holding radio-active matter does not feel any different from ordinary air. The ionized air of a shut-up room with many Bunsen burners alight is close, from the low cooling and evaporative power.

Magnetic storms and Hertzian radiation. It is believed that the sun sends out electrons, or cathode rays, also positively charged atoms of helium or α-rays and γ-rays, there being evidence of radium in the sun's chromosphere. There may be also shot out particles of matter carrying electrical charges. These radiations do not penetrate, but produce electrical effects in the upper atmosphere. The prevalence of aurorae and magnetic storms appear to follow an eleven year cycle of sun spot activity.

[1] Chree, *Proc. Roy. Soc*, 1918, **A. 95**, 210.

There is no evidence that we have our feelings affected by magnetic storms, for they are recognized only through the evidence of the recording instruments.

Living vegetable organisms absorb and conduct electro-magnetic oscillations over a wide region of the electro-magnetic spectrum, not only sunlight and heat but waves of immensely greater length, such as Hertzian radiation. Disruptive discharges between vegetable electrodes and electrostatic effects between vegetable surfaces are easily produced. In its vegetation the earth is provided with efficient 'antennae', every tree covered with foliage being responsive to induced electrical oscillations; hence the danger of sheltering under a tree in a thunderstorm. Forest areas may then exercise an important influence in maintaining equilibrium between the electrical charges of the upper atmosphere and the earth. Comparisons are needed between such areas and great deserts devoid of any vegetation.

If a copper nail is driven into a tree near the top, this can be used as terminal for the study of the potential distribution on the surface of the earth, an earth terminal, consisting of several short pieces of insulated wire sealed at the outer ends and radiating from a common centre, being buried a few inches beneath the ground near the tree. The tree is subject to changes of potential representing the innumerable frequencies required by modern radio-telephony and telegraphy, as well as any other electrical disturbances which may occur on the surface of the earth or the atmosphere above the earth. One or more of the frequencies can be selected by tuned electrical loops suitably connected to the tree electrode, and each in turn studied just as colour screens select a particular component of white light. Connecting it by a highly insulated conductor with the thermionic tube, the particular effects studied can be amplified almost at will.[1]

From the moment an acorn germinates in the soil it becomes a 'detector' and 'receiver' of electro-magnetic waves, which vitalize it and produce in time the giant oak. In its power to multiply plant cells the plant ovum may be called an incomparably good 'amplifier'. There is no evidence of our sensitivity to Hertzian waves, and as the human body insulated by dry shoes cannot act as a receiver like a tree the oppression felt in thundery weather may have nothing to do with the electrical state of the atmosphere.

In cold, dry climates the body can be charged by friction of the feet against a carpet so that sparks can be drawn from it, but there is no sensation of this charge.

A constant current of high voltage passed through the body causes delirium of the heart; a rapidly alternating current is both unfelt and innocuous.

While there is no evidence of the physiological action of electro-magnetic radiation other than heat, light, and X- or γ-rays, it must be borne in mind that the agricultural worker, exposed to the open air, is in this respect in different conditions compared with the factory worker exposed to masses of metal and artificial sources of energy in closed places.

[1] G. O. Squier, *Journ. of the Franklin Institute*, 1919, **187**, 657.

THE QUANTUM THEORY.[1]

Certain physical phenomena cannot at present be explained by the simple wave theory of light outlined here, and this has led to the conception of the 'Quantum Theory' developed largely by Planck and Einstein. Some of the features of this theory are mentioned below.

It has already been stated that ultra-violet light is able to set free electrons from the atoms of metals, and that for each metal there is a critical wave length of a certain frequency which can accomplish this. Now if a constant source of light (energy) is considered, the energy is assumed on the usual theory to spread out equally in all directions, that is to say, the energy reaching unit area in unit time is the same at all points on a sphere with the source as centre. It therefore follows that if the amount of energy given off by the source per second is known, then that which will reach a given area at a known distance from the source can be calculated, and the amount which would fall on and could therefore be absorbed by an electron, at a known distance from the source, may be determined.

From experiment the surprising fact became evident that the energy which the liberated electron possesses is much greater than that which it can have absorbed from the source (the effect is found to be instantaneous, so the possibility of the energy accumulating is eliminated). Further, the intensity of the light has no effect upon the energy of the liberated electron, that is to say, that if the source of light is moved farther away, or what is, of course, the same thing, the electron is moved farther from the light, the energy of the electron when set free is unaltered. Now it is quite evident that the electron must in this case be situated on a sphere (with the source as centre) which is larger than that on which it would lie in the first case, and since the amount of energy reaching the total surface of each sphere per second is the same, that which falls on the electron (whose area remains unaltered) must be less in the second case.

The energy of emission of a liberated electron as given by Einstein's equation is

$$\tfrac{1}{2}mv^2 = hn - p,$$

where m is the mass of the electron, v its velocity, n the frequency of the incident light, p a constant for each metal, and h a universal constant for all metals. p represents the amount of work necessary to get the electron freed from the atom and out of the metal, therefore the energy which the electron must possess immediately before leaving the metal is equal to hn, that is, the energy of any escaping electron must be a multiple of h. It is this quantity of energy hn which has been termed a 'quantum', and the theory has been put forward that the energy of light is not spread over the whole wave front, but is made up of bundles or quanta, and that the electron absorbs one of these and so obtains the necessary energy of emission. Another view is that the energy is in some way stored in the electron, and that the light is able to set it free. The quantum theory may be briefly summed up as follows: 'A simple harmonic ether wave of frequency n communicates energy to matter in finite portions or quanta, of hn ergs at a time, where h is a universal constant which is about 6×10^{-27}. Such a wave cannot be excited by the impact of a body of kinetic energy less than hn' (F. B. Pidduck, *A Treatise on Electricity*, 1916).

The value of h is found to be the same as Planck's constant which appears in other branches of physics, but at present the whole theory of its use is in little more than a hypothetical stage, and no useful purpose would be served by discussing it here. The above outline of the quantum theory has been introduced to indicate upon what lines modern physical theory is likely to develop.

[1] I am indebted to Miss Hargood Ash for this note.

CHAPTER II

The Penetration and Action of Light on Living Organisms.

Little is known precisely as to the action of light on the health of the body. We know that children brought up in sunless streets and courts suffer from pallor and rickets, but these are results of ill-feeding and confinement indoors, a medley of unhygienic factors, one of which is the absence of sunlight. So too with the pallor of prisoners confined in dark dungeons.

Pit-ponies live for years in health in stables at the bottom of mines. In their case the stimulating effect of the radiation energy of the sun may be replaced by that of artificial lights. There are men who carry out night work for years and keep their health. They may be pale-faced not from anaemia, but from lack of sunburn. There is an abundance of nocturnal animals, and animals that live in dark places—owls, moles, the blind fish of cave waters, earth-worms and grubs of all kinds, deep-sea fish. The latter illuminate the cold darkness of the abysmal depth with their own light organs. The light-adapted animal seeks light, and the dark-adapted shade.

There is an Italian proverb, 'all diseases come in the dark and get cured in the sun', and in 'classical' times great store was set on sunning. Pliny built his villa at Naples so as to secure sunshine at different times of day, an excellent means of securing open air and warmth in a sunny climate. The stimulating effect of sunlight on our feelings of comfort and happiness are obvious, particularly in the cloudy British climate. No less obvious is the tiring effect of continual tropical sunlight. It has been estimated that the approximate relative brightness of the sun at the zenith is 300,000 that of blue sky, and 30,000 times that of bright cloud. While the constant change of weather in the British Isles is exhilarating and bracing and the amount of ill-weather impels us to work, it is agreed there is a lack of radiant energy. It requires more winter sun and bright frosts and snow to make the climate ideal.

In Volume I of this report[1] it was pointed out that when the sun warms the skin and breezes keep up a high rate of cooling we get the ideal conditions for health and vigour. Indoors we secure warm skin sensations by stagnant air at the expense of cooling power and with reduction of metabolism.

Fire and artificial light take the place of the sun in giving us radiant energy, and fires are wanted rather than closed stoves, hot-water or steam coils, because in this foggy, cloudy climate we lack the radiant energy of the sun. We want the radiant heat to keep things dry and well ventilated. In North America, Germany, &c., it is not so. Winter sun and snow glare in these countries give abundant

[1] *Medical Research Committee, Special Report Series*, No. 32.

radiant energy and the outside air is cold and dry. Indoors there people can better tolerate stoves and stuffy conditions.

It is estimated that 75–80 per cent. of the sun's energy which enters the atmosphere is obtained when the sun shines at 1,800 metres altitude in the Alps, 50–60 per cent. at sea level, 25 per cent. in a big city, dropping to 15 per cent. when the sun is low and the atmosphere damp.

In open air treatment we want to use the sun warmth to secure grateful feeling while the wind braces up the body. Sun-baths are injurious if they over-heat the subject.

Sunburn and the inflammation and pigmentation which result show how powerfully the rays affect the cutaneous metabolism.

Sunlight acts as a potent disinfector, killing bacteria; the tubercle bacillus for example is one of the powers of darkness.

It is absurd then to shut out the sun from rooms in order to save carpets and covers from fading. People who shut out the sun and wind bring about the fading of their own complexions.

The skin exposed to sunlight is reddened with blood, the blood pressure falls, and the respiration diminishes in rate but increases in depth so that the volume breathed becomes greater (Hasselbach and Lindhard).[1]

The pulse rate of a man sitting quietly in the tropical sun increases on an average 10–12 beats over the shade value. The volume of air respired increases on the average 23 per cent. (Aron).

The mean systolic blood pressure of natives and Americans of the same age was the same in the Philippines, differing little from the average in temperate climates. The pulse rate of whites observed in the Philippines averaged a few beats above the standard 72 per minute. No difference was observed in those of dark or blonde complexion, nor in those wearing orange red underclothing.[2] At rest in the tropics the rectal temperature is much the same as in temperate climates, but rises more quickly on exercise and decreases more slowly after exercise.[3]

Brissoff found that dogs kept in the light ate more and weighed more at the end of a month than others kept in the dark. So with rabbits.[4]

In some experiments[5] designed primarily to test the supposed toxic property of exhaled air, tame rats and guinea pigs were confined together in deep wooden boxes the lids of which were shut down so as to give imperfect ventilation. The animals lived healthily for weeks in an atmosphere containing 1·6–3·8 per cent. CO_2. They were well fed and the boxes cleaned daily; the temperatures wet and dry were taken near the bottom of the boxes.

We tried substituting for the wooden lid of each box one made of glass, so submitting the animals to the stimulus of light and a view of the outside world, in place of dim obscurity. Periods of some weeks first of light and then of dark alternated. The growth in weight of the animals appeared accelerated by light and retarded by dark. The light and the sight of the active world appeared to stimulate activity

[1] Cit. after Graham Lusk, *The Science of Nutrition*, Philadelphia and London, 1917.
[2] W. P. Chamberlain, *Philippine J. Sc.*, 1911, **6**. 467.
[3] Young, *J. Physiol.*, 1915, **49**, 222, and with Breinl, *Med. J. Australia*, 1919, May 3. 10, and 17.
[4] Cit. after M. A. Cleaves, *Light Energy*, New York, 1904, 219.
[5] L. Hill and M. Flack, *Smithsonian Misc. Collections*, 1913, **60**, 23.

metabolism, and growth. Further and more extensive experiments carried on for longer periods are required.

While considering the influence of light on animals, attention may be drawn to the remarkable work of A. E. Rawson,[1] who has brought forward evidence of the different physiological effects on plants of high and low altitude sun. Kei apple plants used as a border to paths in a Cape Town garden died where shadowed for 1 hour 50 minutes from the sun of March mornings. Wherever screened by a distant house, a thick evergreen hedge, or even a single bush, and in full sun at other times, bleaching of the chlorophyll had set in or the plants had actually died. Yellow tomatoes in close proximity but differently screened yield very different crops. The time of screening and when the full sun falls on the plant is of importance to the crop. Rawson has introduced sports of nasturtiums at will in a Hertfordshire garden by the method of selective screening from the sun, and new varieties, he says, have been fixed. Judicious screening ensures that nasturtium plants live on for several years, though normally annuals. In consequence of this longevity persistent screening will transform the pure crimson flowers of one season into pure orange or yellow, and eventually into a form with red spots on one or other of these grounds.

Sterility is almost universal upon any striking change of colour or structure being thus produced, but seeds have been secured and changes transmitted by them.

'There is some peculiar response which plants make to the sun just after it rises, and those plants which have been held in it for only a quarter of an hour when seedlings, have sported subsequently in various ways'. 'Two or three minutes on 31 successive mornings of cloudless sunrises in Natal perceptibly differentiated the individual that received them from the rest.'

Not only colour of flower but form is altered by screening. 'A maximum exposure to low sun is responded to by the appearance of increased yellow or orange; a maximum of the highest sun available develops purple and violet pigments in this latitude, while a plant exposed to the middle sun responds by developing much red pigment.' Middle sun for tropaeolum is that above an altitude of 35°, high sun that above 55°. It is essential that a plant should not be deprived entirely of low, middle, or high sun, for fear of disease setting in, such as occurred in the case of the Kei apple.

It has been pointed out that attacks of plant disease, black aphides, potato fungus, &c. are associated with want of sun. The mechanism of resistance of the plant is lowered by reduced metabolism, while the growth of fungus on the leaves is favoured by damp and absence of sun.

Colonel Rawson points out the variations of light in woods, mountainous elevations, and low-lying valleys, the intense brightness of snow fields and blackness of depths of water. Every stem, branch, leaf, and filament also sets up diffraction bands owing to the interference of rays at their margins. Light then exerts a manifold influence in shaping the growth of species.[2]

[1] Cf. *Hertfordshire Nat. Hist. Soc.*, 1917, **16**, 259. *J. Roy. Hort. Soc.*, 1915, **41**, 1.

[2] The sensitivity of seedlings to light as evidenced by phototropic curvature is maximal in the blue end of the spectrum. Cf. H. Weve, *Arch. néerland. d. Physiol.*, 1920, 4, 243.

It is not easy to explain these results. A low afternoon sun in a tropical country [1] is equivalent to a mid-day sun towards the poles, the sunshine received being a matter of the thickness of the air and the angle at which the rays pass through the layers of the atmosphere. The light from a high sun, owing to less absorption in and scattering by the atmosphere, has a wider range of frequency than that from a low sun. If the direct sun is cut off, the light that reaches the plant from the blue sky will be richer in blue rays; these received apart from heat rays may well affect the metabolism.

Observations which I have made on the surface temperature of flowers and leaves show that a red geranium, shaded at first from the early morning sun, on a fine July day with little breeze, had these surface temperatures:

	Leaf.	Flower.
6 a.m.	14° C.	14° C. shade, dew on plant
		18 sun
9 a.m.		21 shade
		25 sun
11 a.m.	25	26·5 shade
	29·5	31 sun
1 p.m.	32	37 sun

The sudden change of temperature on changing from shade to sun must put a strain on the mechanism of the plant and profoundly affect its metabolism.

I find the surface of dark red and orange flowers 2–4° C. hotter in the sun than that of pale or white flowers, and hotter than the green leaves.

The influence of light has been studied on the growth and reproduction of the mycelia of the algae. It is claimed that a modification of form in the case of one species was transmitted to successive generations with a change from aerobic to anaerobic condition of existence.[2] Comparable effects have been noted in the case of animals. Exposure of the nymphae of 50 species of butterflies to extremes of heat or cold for some hours each day causes a great increase of variants; some extreme enough to be classed as mutants (Standfuss). In the case of beetles mutations were quickly caused by transferring them from Chicago to a dry desert climate and vice versa (Tower). Similarly heat and cold causes mutations in nurse-toads (Kammerer) and white mice (Summer). The susceptible period is the first two weeks of life in the case of mice. After exposing the cold-blooded fruit-fly to abnormal temperature its egg cells on fertilization show variations in the division of the chromosomes (Hoge).[3]

Radiant energy from sun, arc, flame, gas fire, mercury lamp, produce very different quality of feelings. Dark heat waves penetrate the skin and are absorbed in the blood and deeper tissues. Visible rays are to a large extent diffusively reflected at the surface. Those that penetrate are absorbed, and together with dark heat rays excite vasodilation and sweating. Ultra-violet rays are absorbed by the outer layer of the epidermis.

It has been suggested, but there is no evidence, that light from certain surfaces, metals, &c., may have a different physiological effect.

[1] The sun in the tropics has no burning effect till it reaches an altitude of nearly 30°, then the effect rapidly increases.

[2] See *Photobiologie und Phototherapie*, Leredde and Pautrier, cit. after M. Cleaves.

[3] Cit. after Ellsworthy Huntington, *World Power and Evolution*, New Haven, 1919.

Light from incandescent solids and reflected light is polarized. The moonlight has traditionally an ill reputation.

In the case of the dark heat rays, Tyndall placed the charcoal points of an electric arc light in front of a silvered glass mirror, concentrating the rays to a focus about 6 inches distant. He then placed in the path of the rays a cell full of carbon bisulphide solution of iodine, which absorbs all luminous radiations. Platinum foil placed in the focus was raised to incandescence by the invisible heat radiations. A brilliant luminous spectrum may thus be obtained by 'calorescence' of the metal. The eye was next placed in the focus behind a small hole in a screen; no sensation of light and scarcely any of heat was felt although 18 per cent. of the rays pass through the humours of an ox eye.

Powerful as radiant heat rays are—sufficient when focused on them to fuse metals—they have then no stimulating effect upon the retina. Gather them in a focus, and there is nothing to be seen at the place of convergence. The air, too, at the focus would be found just as cold as the surrounding air, could a suitable thermometer be devised to show this, so little are the heat rays absorbed by it.

While the retina does not react to the motion of these powerful waves of low refrangibility, on entering the skin they excite the cutaneous nerves and exert an influence which is spread to all parts of the body. There are infra-red waves which the bolometer detects but not the skin.

The red glow of a gas fire examined with a pocket spectroscope shows red and some dull green. When the hand is held close to the gas fire insufficient light is diffusively reflected from it to be visible in the spectroscope although the hand is almost too hot to bear. The surface of the hand illuminated by incandescent gas and examined by the spectroscope reflects red, green, and some blue and violet. The light reflected from the sunburnt back of the hand indicates some absorption in the yellow which is not visible in the light reflected from the white forearm. An oil lamp is much less rich in actinic and richer in yellow rays than the incandescent gas or electric light.[1] Taking the intensity of red as the unit Abney gives the following:

	Sun-light.	Arc light.	Gas flame.
Red	100	100	109
Green	193	203	95
Violet	228	250	27

By reflection from coloured surroundings the colour of the useful light can be made quite different from that of the source. Thus daylight can be imitated by appropriate choice of colours of a reflector surface, or by an appropriate filter.[2]

Using a thermopile enclosed in a water-jacket, I measured the diffuse reflection of light from the skin of the hand, placed in front of a conical opening in the jacket, so that the reflected light fell on one set of thermo junctions, while the other set was kept at the temperature of the water-jacket. The source of radiant energy was placed about 2 feet away except in the case of the large arc, which was 9 feet away.

[1] M. A. Cleaves, *loc. cit.*
[2] Luckiesh, *Abstr. Bull. Nela Res. Lab. U.S.*, 1919, 223.

The galvanometric arrangement was not a sensitive one, but sufficed to show the difference of effect between different sources of energy.

Using a small copper arc light and suitable colour screens to secure only light of short length and ultra-violet rays, there was no observable reflection of these from the hand on to the thermopile. When the light was allowed to fall directly on to the blackened face of the thermopile it only gave a slight deflection, the heating effect being extremely small. A tungsten filament lamp gave an increased deflection of 1 division of the scale from the palm of the hand when the light was 6 inches from it. When the light of a mercury lamp was allowed to fall on the hand there was no measurable deflection. The light of an ordinary incandescent gas-mantle, concentrated by a lens and passed through a water screen, gave no measurable deflection from the palm; the naked light gave a deflection of 3 divisions of the scale when brought near to the palm.

A large arc lamp and sunlight gave a big deflection, 10 divisions of the scale or more. This deflection was not due to increased surface temperature, as shown by the immediate effect of screening off the light. Sufficient light was diffusively reflected from the skin to illuminate the black surface of the thermopile, but in comparison with a polished metal surface the reflection from the skin which reached the thermopile was small. That from the polished surface at once sent the needle off the scale.

Using an arc light to illuminate the forearm, and a water screen to cut out the heat rays, I found that a coloured screen which cut out all the red, orange, and a little of the green lessened the thermopile deflection by 3 divisions of the scale in 42 seconds, a green screen by the same amount in 24 seconds, and a blue screen in 22 seconds. The green-blue rays were reflected from the skin, then, more than red.

When the hand was exposed to the red glow of a gas fire, the red rays appeared to be mostly absorbed, not reflected.

Exposure to strong light has an excito-motor action on unicellular animals. The amoeba contracts. The fact may here be recalled that the rods and cones in the retina of the eye also contract on exposure to light. Small animals confined under colour screens avoid the blue and seek the red light (Finsen). Tiny crustaceae flee from ultra-violet rays; they first exhibit great agitation, and finally become immobilized under their influence. Marshall Ward[1] observed the destruction of anthrax bacilli by the blue end of the spectrum; the ultra-violet rays were most destructive.

Reactions are set up in living cells by radiant energy which may be more or less extensive and prolonged and not proportional to the initial effect. Nothing is known as to the nature of the initial effects. Catalysts may possibly be set in action, but there is evidence of a reduction process taking place in the living substance under the influence of light.[2]

Egg white after exposure to the mercury vapour lamp is precipitated on immersion in a 0·1 per cent. calcium chloride solution. An abnormal quantity of this salt increases the sensitivity of the skin to sunburn,[3]

[1] *Proc. Roy. Soc.*, 1892, **A. 51**, 183. [2] Bayliss, *loc. cit.*, 571.
[3] See p. 29, Part I.

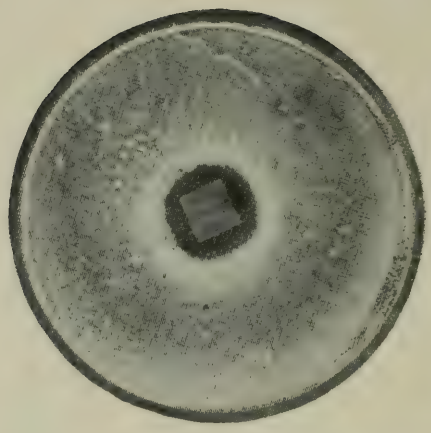


FIG. 10. Effect of silver in checking growth of *Photobacterium phosphorescens*. Dewar.



FIG. 12. Photograph of a culture of phosphorescent bacteria after exposure to ultra-violet light. The culture was screened by a plate of tin except where a cross was cut out of the tin. Dewar.

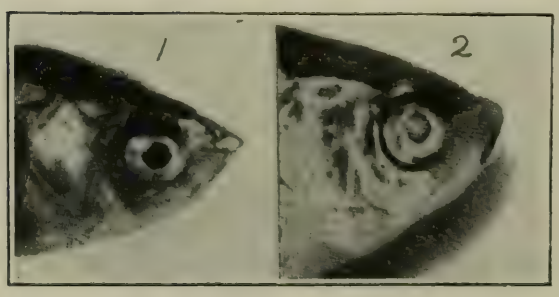


FIG. 13. 1. Fish living in tap water exposed to ultra-violet light for two six-hour periods. 2. Fish living in 0·1 per cent. sodium silicate exposed to the same. Burge.

of the eye of a fish to the mercury vapour lamp. A sodium silicate solution similarly sensitizes the eye of a fish.[1]

In certain cases ultra-violet rays may produce almost instantaneous reaction.[2]

As the ultra-violet rays are capable of killing bacteria and protozoa in water in a few seconds, water is sterilized very readily by a quartz mercury vapour lamp. In the case of *Bacillus coli* the water need remain in the apparatus exposed to the lamp for only 5 seconds.[3]

To produce the lethal effect of light the mercury lamp is largely used as the source of ultra-violet rays, but electric sparks, produced by an induction coil between various metallic terminals with a condenser in parallel, emit light of shorter wave lengths and of greater intensity.

The aluminium spark in air emits a group of strong lines near λ 1300 (Ångström units); these are very effective in ionizing air.

The discharge in hydrogen at a pressure of 1 to 5 mm. Hg is extremely rich in light of very short wave length, and far more potent than the mercury lamp. The best pieces of fluorite may be transparent to waves as far as λ 1250, the quartz glass of a mercury lamp transmits only as far as λ 1849, common soda glass 2–3 mm. thick as far as 3300 (when thin, 0·2 mm. or less, as far as λ 2500). A column of water 1 cm. long cuts out all wave lengths shorter than λ 2200. Air has an absorption band due mostly to absorption by oxygen, extending from about λ 1270 to λ 1760.[4]

Taking rays of equal energy as measured by a thermopile, a wave length of 280 $\mu\mu$ kills rotifers in 15 seconds, and a wave length of 440 $\mu\mu$ in 4–5 hours.

While then all rays visible and ultra-violet of sunlight or the arc light exert a lethal effect on protoplasm on long enough exposure, intense germicidal action is produced by certain rays, e. g. between 2960 and 2100, the maximum being between 2800 and 2540 (Ångström units). The germicidal action is best shown by exposing culture plates uniformly sown with a microbe to the required rays, using a cadmium arc with quartz spectroscope and fluorescent screen, developing the plate afterwards and using it as a negative for photographic reproduction. The developed colonies are sharply marked out from the area where the rays have had their lethal sterilizing effect.[5]

The penetrating power of these effective rays is so small that they are completely absorbed by 1/10 mm. thickness of living substance, e. g. by the horny layer of the epidermis; for example, if the skin taken from a blister is spread on a quartz plate and interposed between the spectroscope and a photograph plate, complete absorption is found of the spectrum of the arc at and beyond 3260. The skin from a blister or the thinnest film of agar protects bacteria from the intense action of the short rays (Barnard).

[1] W. E. Burge, *Trans. Illum. Engineer. Soc. U.S.*, 1915, **10**, 932.

[2] W. T. Bovie, *Bot. Gaz.*, 1915, **59**, 149.

[3] M. Foulds, *J. Roy. Army Med. Corps*, 1911, **16**, 167; J. C. Thresh and J. F. Beale, *Lancet*, 1910, ii. 1849; W. P. Chamberlain and E. P. Vedder, *Philippine J. Sc.*, 1911, **6**, 383.

[4] A. H. Hughes, *Photo Electricity*, Cambridge, 1914, 136.

[5] Barnard and Morgan, *Proc. Roy. Soc.*, 1903, **72**, 126; Browning and Russ, *Proc. Roy. Soc.*, 1917, **B. 90**, 33.

The absorption by a 2 per cent. solution of rabbit serum in physiological salt solution in a layer 1 cm. deep was 70 per cent. for wave length 3131, increasing to practically complete absorption for wave length 2301.[1]

The rays which can affect the living tissue are only those absorbed by it, and are those with the same frequency of vibration as molecules of, or within, the living substance.

A tumbler into which that note is steadily sung which sets up resonance vibrates and may finally shiver into fragments. Incessant vibrations of the stroke of an engine and violent shocks are destructive to germ life. Agitation for 10 hours with glass beads sterilizes a culture. So, too, the high frequencies of ultra-violet rays overthrow the molecular structure and kill protoplasm.[2]

Mr. J. E. Barnard tells me there is some indication that the lethal effect is intensified at periodic intervals along the spectrum, just as in audition the octaves of musical notation mark recurring periods of similar sensation.

As already pointed out, it is only red-yellow light rays that have any great power of penetrating the skin. The curve of the penetrating power starts from the extreme ultra-violet, where the penetrating power is extremely small, and rises to a maximum in ultra-red. It has been estimated that about 22 per cent. of the red-yellow rays pass through the flushed rabbit's ear, only 1 per cent. of the blue-violet, and none of the ultra-violet.[3]

Mr. Barnard gave me the opportunity of testing the penetration of the skin by the ultra-violet rays, using the cadmium arc light with quartz prism and fluorescent screen. A thin flake of the horny layer of the skin cut from the palm was soaked in glycerine and then passed along the spectrum from red to ultra-violet. The depth of shadow of the flake showed the penetration. In the red there was considerable penetration. The shadow became darker and darker passing to the violet and black in the ultra-violet. A dry flake was more opaque all along the spectrum: thus the rays which possess the greatest energy for producing chemical action do not penetrate the horny layer of the skin. It is, then, the visible rays which penetrate and produce sunburn. A preparation of the pigment melanin was prepared from ox eyes, and a film of this pigment made on a quartz slide. On passing the film along the spectrum the opacity increased from red, which penetrated, to violet and appeared absolute in the ultra-violet. A thick black film absorbed all the rays. A moderately thick film let red through alone.

A thin layer of melanin or chlorophyll protects the moist hand from burning with a sun-glass. If the melanin is spread in water, the rays absorbed and converted into heat cause evaporation. Such a layer affords a perfect protection; such is the case with the sweat-moistened skin of the negro (W. Lowe).

A glow-lamp placed in the mouth of a white man, sitting in a dark room, shines through with a red glare, not so in the case of a black negro. The yellow Mongolian lets less light through than the white.

[1] M. and V. Henri, *Compt. rend. Soc. de biol.*, 1912, **73**, 319.

[2] The action of these rays is antagonized by a beam of visible, still more by a beam of infra-red, rays. J. L. Pech, *Compt. rend. Acad. d. sc.*, 1920, **170**, 1246.

[3] G. Busch, cit. by V. Noorden, *Pathology of Metabolism*, 1235 (Heinemann, 1907).

Finsen exposed the arm for one hour to an arc light, and protected parts with screens of (1) white, (2) coloured glass, (3) rock crystal, (4) Indian ink. All protected the arm from damage except the rock crystal. In the unprotected parts there resulted erythema, desquamation, and deep pigmentation. As a result of a subsequent exposure only the white parts protected in the first experiment suffered a dermatitis. The pigmented parts were completely protected.

Some red-haired people have a thin delicate skin which does not pigment and peels after every exposure to sun.[1]

By means of a fluorescing solution the lethal power of light may be increased a thousandfold, the radiant energy being thereby changed into chemical energy. There is thus produced vesicular erythema which affects cattle and sheep fatted on buckwheat. The spots covered with black hair are not affected. Buckwheat contains a fluorescent material, and the disease seems to be dependent on a sensitizing of the skin by its means. By the use of suitable sensitizing materials the red, yellow, and green rays, which penetrate deeply, may be made chemically active. Thus by the use of the dye erythrosin bacteria may be sensitized to orange and yellow frequencies and killed by light under the skin.[2]

Examples of sensitization by certain salt solutions have already been cited. It has been suggested [3] that in blackwater fever there may be a sensitization through destruction of the red corpuscles either by toxin or quinine.

Sensitization may be produced by nutritive changes. Thus, in cases which occurred at a time of intense insolation, war oedema, produced by malnutrition, was localized in the exposed parts, face, neck, and back of hands.[4] The oedema was probably due to the lack of protein, for rats fed on a carrot diet with a low protein value get oedema of the cavities of the body and sometimes of the chest and fore-limbs —an oedema cured by the addition of casein to the diet.[5]

The pocks of small pox are mitigated by protecting the patient with red curtains.

There is a congenital defect of the skin known as xeroderma pigmentosum, an excessive sensitivity to tropical sunlight, resulting in freckle-like pigmentations and indolent ulcers.

The ordinary phenomena of inflammation result in the tadpole's tail on exposure to intense light. So too in the skin.

Severe conjunctivitis may be set up by exposure to sun, snow glare, or arc light. The cornea absorbs all rays shorter than 295 $\mu\mu$. In welding metal with the arc it is important to use coated metal electrodes, the light and heat so produced being much less than is the case with the carbon arc. Crooks introduced tinted spectacle glass which cuts out the lethal rays. It is advisable to wear a yellow-green glass of minimum colour, opaque to ultra-violet rays, and to supplement this by neutral tint or smoke glasses to reduce brightness to a safe value.[6]

[1] Surgical tuberculosis in such is not benefited by heliotherapy. H. Gauvain, *Tubercle*, June, 1920.

[2] Dreyer, *Mitth. Finsen's. Med. Lichtinstitut*, vii, 1904.

[3] In a letter by Mr. Douglas Reid to the writer, see p. 55, note 3.

[4] Rancke, *Berl. klin. Wochenschr.* 1918, **55**, 1238.

[5] E. A. Kohman, *Proc. Soc. Exper. Biol. and Med.*, 1919, **16**, 121.

[6] Luckiesh, *Abstr. Bull. Nela Res. Lab. U.S.*, 1919, 301.

Sun on snow produces sunburn more quickly than sun on rocks or sun in the hot valleys. Those who spend their winters in Alpine resorts acquire a curiously brown colour in the parts exposed to light.

'R. L. Bowles [1] painted his face brown, and on a brilliant day, with eighty other people staying in the hotel, ascended the Gorner Grat at a time when there was much snow. In the evening every individual who had been up the Gorner Grat (himself excepted) was smarting under the effects of sunburn. Twenty people who had kept near the hotel, although they had been fully exposed to the sun, were quite unaffected. A lady wearing a red and white striped shirt on her return from the snow suffered from sunburn on her neck and arms. She was burnt in stripes; beneath the red stripe the skin was perfectly white, whereas under the white stripe it was everywhere crimson; the white

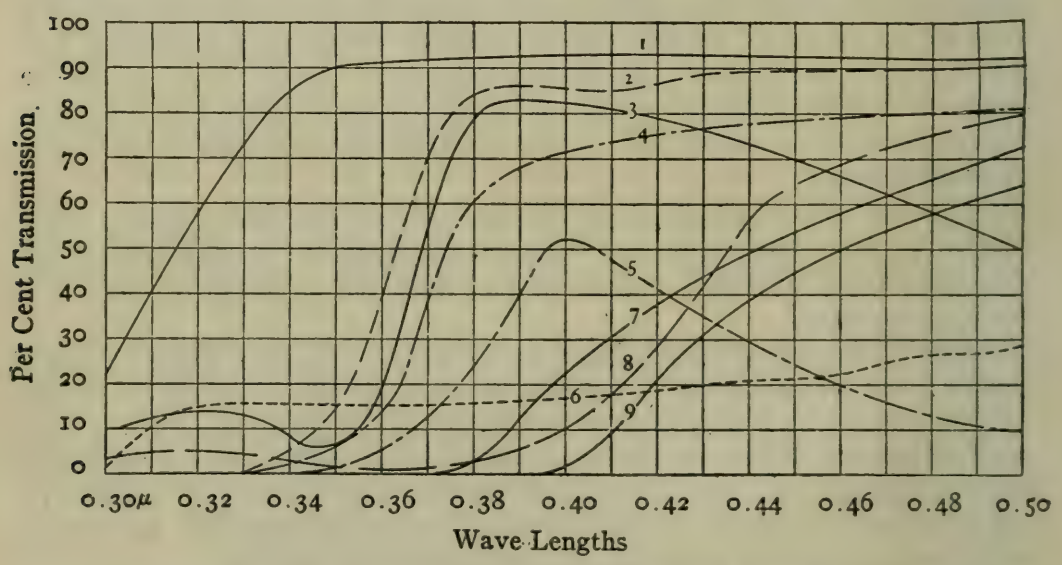


Fig. 14. Transmission-curves of various glasses—

1 Clear lead glass	4 Light amber	7 Medium amber
2 No. 0 Smoke	5 No. 7 Smoke	8 Euphos
3 Amethyst	6 No. 6 Smoke	9 Akopos

stripe was not so closely woven as the red.' Thickness or closeness of weaving is required to prevent penetration. There is no virtue in red. White garments reflect most light.

All along the north of Africa the inhabitants blacken themselves round the eyes to avert ophthalmia from the glare of the hot sand. In Fiji the natives, who are in the habit of painting their faces with red and white stripes as an ornament, invariably blacken them when they go out fishing on the reef in the full glare of the sun. In the Sekhim Hills the natives blacken themselves round the eyes with charcoal to palliate the glare of a tropical sun on newly-fallen snow.[2]

Sunburns are then produced alike in the heat of a desert or in the cold of the Alpine glaciers.

The third day after exposure of the skin to the sun there are found, on microscopical examination of the skin, a slight separation of the horny cells, and evidence of nuclear division in the rete mucosum, with

[1] *Brit. Med. Journ.*, 1894, **2**, 694.
[2] R. Abercromby, *Nature*, April 15, 1886.

vaso-dilation, slight oedema, and increase in the number of leucocytes in the derma.

On the eighth day there is evidence of active nuclear division of the cells of the derma which effect repair, and great vaso-dilation. The reaction compared with a heat burn is slow in appearance and in disappearance. After exposure to the arc light the swelling of the cells of the rete mucosum and formation of a vesicle may begin in the second hour.

Finsen showed that a more active cutaneous circulation follows the erythema produced by light and lasts some time. The hair grows more in summer light than in winter; thus animals put on a coat ready for the winter. The erythema of, and increased metabolism in, the skin set up by sunlight may be most important in keeping up the immunity to disease, acting like a vaccine when injected subcutaneously, stirring up the mechanisms which defend against cutaneous injury.

The Finsen light treatment of lupus does not directly kill the infecting tubercle bacilli, for the active rays do not penetrate sufficiently to effect this, but acts as an irritant to the tissues resulting in congestion, increased outflow of immunizing lymph, and increased phagocytosis. Since dilation of the superficial capillaries is the immediate result of the exposure of a white skin to the sun, the effect of absorption of the visible rays, such as penetrate the epidermis, falls mainly on the red cells.

The blood serum fluoresces, so light must have a chemical action on it.

Oxy-haemoglobin in strong concentration absorbs all but the red of the visible rays. In weak concentration it shows the well-known selective absorption bands. The infra-red rays are also absorbed to a considerable extent by oxy-haemoglobin. Light produces a change in the equilibrium between oxygen, carbon monoxide, and haemoglobin, a photo-chemical reaction, but does not affect the equilibrium between oxygen and haemoglobin.[1]

An hour after exposure to a light bath an increase in the number of red and white corpuscles was found by Manté; the percentage of polynuclear and large and small mononuclear cells was raised.[2]

Red corpuscles succumb and undergo haemolysis twenty-four hours or so after exposure to radiation from a mercury lamp.[3]

The complementing power of serum, tested by haemolysis, is decreased from excessive action of light. Ferments likewise are weakened or destroyed.[4]

There is enough evidence then to show that the sun may have an important influence on the blood, which it brings to the surface of the skin. The pigmentation which follows sunburn protects the blood from excessive action of light.[5]

'There is no evidence that anaemia is caused by the tropical climate

[1] Hartridge and A. V. Hill, *Proc. Physiol. Soc.*; *J. Physiol.*, 1914, **48**, 11.

[2] Cited by P. Krause, *Handbuch der gesamt. Therapie*, Penzolt and Stintzing, 1911, **5**, 383.

[3] Haematoporphyrin injected under the skin of a white rat sensitizes it so that it dies on exposure to the sun. Haussmann, *Ztschr. f. Biochem.*, 1910, **30**, 276.

[4] V. Baroni and C. Jonescu-Mihaiesti, *Compt. rend. Soc. de biol.*, 1910, **68**, 393; S. C. Brooks, *J. Med. Research*, 1918, **38**, 345.

[5] C. Neuberg, *Ztschr. f. Krebsforsch.*, 1904, **2**, 171; H. Huber, *Arch. f. Hyg.*, 1905, **54**, 53; C. Packard, *J. Exper. Zool.*, 1914, **16**, 85; B. Scheube, *Diseases of Warm Countries*, Philadelphia, 2nd ed., 1903, 150.

alone. A certain number of Europeans take on a peculiar whitish colour even in cases in which the blood examination does not reveal any sensible decrease in the amount of haemoglobin.' (Castellani & Chalmers.)

After twenty months' Philippine service healthy American soldiers show an average of 5,200,000 red cells per cm. and an 89·6 per cent. haemoglobin reading.[1]

Degenerative atrophic changes are said to result in the skin of white men after long periods of work in tropical climates. Rodent ulcer and epitheliomata of the face are common in Australia in the white, rare in the coloured races.[2]

The X-rays or γ-rays of radium, just as the ultra-violet rays, cause ionization accompanied by chemical action. They decompose water into hydrogen and oxygen, and cause the combination of these gases with formation of water, produce ozone in air, precipitate globulin made electro-negative by ammonia, dissolve the opalescence of globulin made electro-positive by acetic acid, decompose organic matter such as the grease of stop-cocks, and have a lethal action, severe exposure resulting in acute inflammation and necrosis. Hair-bulbs and sweat glands may be destroyed, muscle and nerve fibres made to degenerate. A proliferation of the endothelium of the small capillaries occurs and the lumen may be occluded thereby.

The inflammatory changes, properly controlled, have a beneficial effect in chronic cases of ring-worm, rodent ulcer, and lupus.[3]

The X-rays have also been used with advantage on the thyroid in cases of exophthalmic goitre, on fibroids in the uterus, and on bone marrow and lymph glands in cases of leucocythaemia.

Pigment does not protect from the X-rays or the γ-rays of radium. Acting on the testicle they have a sterilizing effect, so that operators protect themselves with lead aprons. Acting upon the ovary they produce amenorrhoea.

Repeated exposure to X-rays may produce a dermatitis ending in cancer of the hands, the fate of several early operators who knew nothing of this hidden danger.

[1] Chamberlain, *Philippine J. Sc.*, 1911, **6**, 483.

[2] N. Paul, *The Influence of Sun-light on the Production of Cancer in the Skin*, London, 1918.

[3] The X-ray owes its activity to the electrons which it produces when arrested. Its use is to plant the action deeper in the body (Bragg). Cf. C. W. C. Kaye, *X-rays*, London, 1918, 3rd ed., 172.

CHAPTER III

The Colour of the Skin and Racial Adaptation to Climate.

The colour of the skin of different races varies from the very pale reddish brown of the so-called white races exposed to weather through all shades of yellow or red brown to olive and chocolate, which may be so dark as to look black. The question has been much discussed as to how far pigment is an adaptation to insolation, including reflection from sand or snow-fields, and how far it is a protective adaptation for concealment in forests, &c. The evolution of the white and negro races may go back to a vastly remote period of time.

Sir Harry Johnston in his *Negro in the New World* sets forth the classification of the subspecies of man thus :—

1. Australoid, nearest to the palaeolithic man of Europe and North Africa, and to the possible parent thereof—*Homo primigenius*—the man of Neanderthal.

2. The Negro.

3. The Caucasian or European, possibly descended in a direct line from the Australoid or basal stock.

4. The Mongolic or Asi-American. Intermingling of 1, 2, 3, 4 has produced the Polynesian, 2, 3, and 4 (4 predominating) the Japanese. The Amer-Indian, he says, are mainly descended from an early branch of Mongolic mixed with Proto-Caucasian. Hence they mix with whites and the hybrids become as whites. The negro blood, on the other hand, pigments the hybrids.

Woodruff regards the negro's pigment as a climatic adaptation to the heat and humidity of the forest, and W. Lowe as an adaptation to insolation.

Freer, on the other hand, thinks the colour of the negro is protective—he could always seek the shade as the monkey does, there is then no reason to attribute it to insolation.

J. W. Buchan correlated the colour and texture of the skin with the moisture of the atmosphere and the sunlight of different climates, which he classified as arctic, moist temperate, dry temperate, moist tropical, and dry tropical. In the arctic climate the skin is exposed to great cold, and the absolute humidity of the air is very low. A rough, thick skin and a thick layer of fat are required to protect the body from heat-loss ; such are found most frequently among polar tribes. The skin is darker among these than among blonde Europeans because, he says, of the light from the perpetual snows. No men can, however, expose the naked body to light less than the Esquimaux. They must go clothed out of doors, and only strip in their snow huts wherein they closely cuddle for warmth and steam together. If then their colour is due to light, it must be brought about by light acting on the small part of the face exposed, and the eyes. It seems more probable that a yellow Mongolian or Amer-Indian race spread up to the polar regions.

In the moist temperate climate the air is nearly saturated, and the rays of the sun falling obliquely through a moisture-laden atmosphere lose much of their light and heat. Neither pigment nor a thick epidermis is needed. Fair white complexions are produced, says Buchan, in such a climate, particularly where there is shelter from cutting winds. Big men, heavy weights, can exist in such climates in comfort. Much adipose tissue can be borne, as this is a protection against cold mists of winter, and not a disadvantage in the cool summer.

In the case of plants grown in moist air the formation in the leaves of nitrogenous products and starch is more active, there is less chlorophyll, the stomata are wider and more numerous, the cutis thinner, the vessels wider and their walls thinner, and often the spines of the leaves are undeveloped. Similarly, a moist climate makes the skin thinner, softer, and more delicate, and gives the beautiful complexion of, for example, the Irish girl.

In a dry temperate climate, such as found in the prairies of North America, the summers are hot and the winters cold. A thicker epidermis and some pigment is required. To face the summer heat the adipose tissue requires to be restricted, men are thinner, hence the difference between John Bull and Uncle Sam (Lusk).

A moist tropical climate is found in dense forests with a steaming enervating atmosphere, a high temperature, and little wind. Pigment is needed not to protect from light, but to conceal the naked man. A thin skin is required to promote heat-loss by evaporation and convection. The tribes of the Amazon regions, the Bornean jungle hunters, and the pygmies of tropical African forests are of the colour of old ivory rather than brown.

In dry tropical climates dense pigment and a thick scarf skin are needed to protect from light. In rainless Nubia the inhabitants, says Buchan, whether of Semitic, Hamitic, or negro stock, are alike black; south of the great desert those of the coast and central depression are black; those of the more or less elevated rim where moisture is deposited fairer. There are black men in Africa, India, Peru, Australia, and some of the adjacent islands, because these countries all have pronounced dry seasons.

The colour of each race is now so fixed, says Buchan, that climatic influences change it very slowly. 'Wherever there are reasons to believe that there has been a settlement of Germans or Scandinavians the complexions are to this day comparatively fair. The nine centuries that have elapsed since the Northmen settled in Normandy have not made their descendants as dark as the neighbouring Bretons; nor have thirteen hundred years made the West Saxon of Somerset and Gloucester similar in complexion to the Welshman of Glamorgan and Caermarthen.

'The Spaniard of South America, the Englishman of Virginia, the Frenchman of Quebec, seem to be precisely the same physically as the Spaniard of Spain, the Englishman of England, and the Frenchman of France. If the white race darkens within the tropics, or the negro blanches under the influence of frost, either process is very slow.

'Side by side in the same country, as for example India, we find races of differing colour, who apparently have occupied the same soil

for many centuries, the European brunette, the blonde Circassian, and the yellow Mongol. On the equator itself we have the African negro, the brown Malay of Borneo, and the yellow Tupi of the valley of the Amazons: north of the blonde Russian is found the yellow Samoyede, south of the brown men of equatorial Sumatra and Java live the blacks of Australia. The Scandinavian Peninsula is the seat of the fairest population in the world, and the blonde centre is probably somewhere in its southern half. In every direction, north, south, east, or west from this central point, the proportion of blondes decreases and that of brunettes increases, but the blondes penetrate, if in small numbers, everywhere into the tropics.

'In America the aborigines of Alaska are almost white, the Californian and Arizona Indians are dark brown; the Tupis and Guarenis that occupy the valleys of the Orinoco and the Amazons are yellow; the Peruvians and the aborigines of the La Plata and Patagonia are brown. The Charruas, who live near the mouth of the La Plata, are sometimes described as black. Blondes occur from the Arctic ocean to the Sahara, and from the Atlantic to the Indus, in greater or smaller numbers; and occasionally beyond these confines among the Chinese or Coreans, or even the Indians of North-West America, individuals may be met with, of pure blood, who exhibit light eyes or fair hair.'

Dr. A. C. Haddon writes to me as follows:—

'Heat and moisture (of a tropical forest) do not induce a black skin; the Brazilian and Bornean tribes prove this, unless it be urged that they have not been there long enough, but of this there is no proof. Absence of direct prolonged sunlight may have something to do with the lighter colour of some jungle tribes, for example the nomadic jungle hunters of Borneo, so too the pygmies of the tropical African forests have a general colour of old ivory—orange rather than brown—but the bushmen of the steppes of South Africa are yellow skinned, as are the Hottentots (= Bushman, Hamite, Bantu mixed.) The Bantu even of South Africa vary a good deal in colour (they are mainly Negro, Hamite mixed). In the Pacific, under apparently identical conditions, you find dark chocolate-brown, some even black, Melanesians, or light brown among the Polynesians, some practically like South European brunettes. The Melanesians are a Papuan Proto-Polynesian mixed race, and the latter were a mixed people from Indonesia: probably one element of these came from the valley of the Ganges. The true Papuans (i. e. those unmixed with Melanesians, &c.) are so far as I know invariably dark chocolate-brown. Light reflected from the ground may have some effect, but I doubt if it covers all the tropical evidence, i.e. those living in jungles or mud swamps—but it may do so. Anyhow, the Bedouin of Arabia are not black. The Kabalish of the Egyptian Sudan often are very dark—but we know there is negro mixed with them. As to snow, the Eskimo are by no means black, nor yet very dark, some say yellow, others brownish or reddish yellow.'

Professor W. H. Jackson writes: 'Animal blackness, I think, is an adaptation to environment in which animals seek their food, e. g. forests, or to the time at which they seek it, e. g. by night. But then how about black horses and cattle? One would have to hunt bionomic

books on coloration to find out if the feral race is ever black. The whole question is crossed by the facts of how colours fade out as light fails, e. g. on a bright evening a certain moment comes when a scarlet geranium looks black, and I have noticed how little even a bright moonlight shows up scarlet flowers. The sloth which dwells in forests is not black; but before it is cleaned it is often green by the growth of algae in the thick fur in the damp atmosphere.'

In considering the influence of a tropical climate on the white man, such factors must be considered as excessive heat and humidity, and consequent lack of exercise, want of sleep, sun radiation, improper diet, alcoholic and venereal excesses, and, more important than all, infections with pathogenic organisms peculiar to the tropics.

According to J. S. Mackintosh,[1] for their vigorous health the blondes require the climatic conditions which are found in the regions bordering on the Baltic sea—plenty of sunlight not associated with tropical heat or great humidity. The most popular holiday resorts of the ruling clans, among whom many blondes are found, are the East Coast, hill country, and high Alps in winter. The open-air life and dry climate of Australia, the South African veldt, and Canada provide a favourable environment for blondes. He cites Tacitus as saying: 'it is well known that none of the Germanic nations inhabit settlements. They dwell scattered and separate, as a spring, a meadow, or a grove may chance to invite them. Their villages are laid out, not like ours in rows of adjoining buildings, but every one surrounds his house with a vacant space.'

Blondes are said to be more susceptible[2] to acute rheumatism, tonsillitis, osteo-arthritis, and catarrhs—to infections spread by confined indoor life. But very little seems to be known concerning racial susceptibility.

Sir Clifford Allbutt recently asked these questions: 'Do the children of the darker people in towns outlive the fair? And if so, why? Are the darker people gaining in numbers upon the fair?

'Hysteria is, or was, rare among our Yorkshire folk. Why?

'What is the extra vigour of cross-breeds?'

'We have to learn', he says, 'how diseases vary with the systems of external relations, the conditions of time and place, of season and soil, race and temperament.'

The rise of industrialism, together with greater facilities of transit, has brought about the disintegration of rural communities, the dislocation of rural types, and their migration into cities, where indoor life resembles that in the tropical shade conditions. The blonde appears to be more out of adjustment there than the brunette, while the Jews are well adapted by racial experience.

It is probable that the manners and customs of life transmitted by parents to children are chiefly responsible for the ill adaptation. Diet, exercise, &c., require to be regulated in quite different ways to suit city experience, and the children of parents who have migrated to the city neither learn nor teach to their children these ways. Welfare-instruction, and a disciplined town life may effect a great improvement.

[1] *Brit. M. J.*, Feb. 25, 1911, and Aug. 2, 1913.

[2] Shrubsall, *St. Bart. Hosp. Rep.*, 1903, **39**, 63; J. S. Mackintosh, *Brit. M. J.*, Oct. 8, 1910.

C. E. Woodruff[1] in a series of publications has strenuously upheld the view that blondes, who he says are the most virile and governing class of the world, cannot stand a tropical climate. Since prehistoric times the northern white races have been over-running the South of Europe, but Southern Europe still remains coloured.

In Italy the Lombards still flourish north of 45°, and in Spain the Goths survive in the Northern mountains. The northern third of France is more Teutonic than the southern third of Germany. Blondes survive in Canada, few in New England, none in Louisiana.

He maintains that pigmentation of a race is in direct proportion to the intensity of light in the country to which the race is adjusted. That just as the blondes are exhausted by the tropical sunlight, so the negroes die out in the colds of the North (U.S.A.). There is a constant drift from the South to replace them.

Negroes, says Woodruff, demand a room temperature over 80° F., and the Pullman car attendant cannot understand the complaints of his passengers when the car is so comfortable to him. The wide nostril of the negro is, he says, evolved to admit the rarer warm air of the tropics. The negro cannot narrow his nostrils to warm the cold air of the north! One, however, accompanied Peary to the North Pole.

'In the streets of Port-au-Prince,' says Sir Harry Johnston, 'in a temperature 95 degrees in the shade and something under boiling-point in the sun, you may see Haitian statesmen cavorting about in black silk hats of portentous height and glossiness, with frock-coats down to their knees, and wearing lemon kid gloves.'

There were, as reported in 1914, 9,827,763 negroes in the United States, of whom 8,746,709 or 89 per cent. were in the Southern States.[2]

On the other hand, Sir Harry Johnston says 'it is a mistake to assume the negro is fond of the sun's heat or of a muggy atmosphere. He endures these discomforts better than we do, and is physically much better suited than we are to exist in a perpetual Turkish-bath atmosphere. But just as his ideal of beauty is the same as ours, so probably is his ideal climate. No region of Africa, however elevated, has proved too cold for negro habitation. In North America the negro probably stands cold as well as the white man does, and in North America and South Africa his race is as much stimulated to better physical and mental development by a temperate climate as is the case with the Caucasian.'

A striking example of acclimatization to cold is the Coypu rat which inhabits tropical Central America. Transferred to New York Zoological Gardens this animal goes abroad as usual in winter when the temperature is 2–6° below zero, dives through a hole in the ice, remains under the cold water some minutes, and nibbles food on the ice.[3]

According to Woodruff 'heavy weights' of the north are killed off in the tropics, while small brunettes cannot digest enough to keep warm

[1] Cf. *The Effects of Tropical Light on White Men*, 1905; *Expansion of Races*, 1909.

[2] E. Wilkinson, *Proc. Roy. Soc. Med.*, Epidemiol. Section, 1914, 7, 195.

[3] Bull, *New York Zool. Soc.*, 1918. Rats no less than men stand enormous variations of climate, e.g. −60° C. out of doors in Siberia (plus the cooling influence of wind), 40–45° C. in a room in Senegal.

in very cold climates where the 'heavy weight' is comfortable in his shirt sleeves. He concludes that climatic factors eliminate migrants chiefly by killing the infected ones who would recover in an environment like the ancestral one. His contention is that permanent colonization of the tropics by whites is impossible ; an everlasting immigration must take place there from the breeding-grounds in north-west Europe.

He cites W. Wallace as saying that it is impossible to acclimatize any breed of domestic animal to a climate markedly different from that which evolved it. He says breeders are under the permanent necessity of constantly importing brood animals to keep the stock from an otherwise inevitable deterioration. A new breed must be evolved out of survivors and by crossing to meet the new climate.

As to the question of domestic animals, a breeder tells me the best Arab horses, even white ones, have pigmented skins. So too, in the case of the Indian cattle, while the prevailing colour of the majority of the Indian breeds is white, or grey with occasional black points, their skin is brown and pigmented, and their fat a deep yellow. This in a minor degree is the case with the white Italian cattle, which my informant, Mr. A. F. G. Ellis, has for that reason also imported into Jamaica. 'In Italy the strongest and most favoured points are the black pigmentation of the skin around the eye, muzzle, tongue, and scrotum, &c., in the white breeds. The sugar estate owners in Jamaica much appreciate the Indian cross of cattle, and are much adverse to white-haired (and white round the eye) British-bred cattle. They soon recognized that the white-haired Indian cattle on account of their dark skins had none of the sensitiveness of skin of the white-haired British breeds of cattle like Shorthorns and Herefords—a sensitiveness which makes them unsuitable as draught oxen.'

According to Woodruff white horses rarely get sunstroke.

The Channel Island cattle have brown pigmented skins and yellow fat, and Mr. Ellis suggests that these cattle were brought there from the East by the Phoenicians. The Italian breeds, he says, are recognized in Italy to have their origin in a cross of Hun and Indian hybrids.

'The black skin round the eyes of cattle seems to keep the eyes healthy and free of sores—less sensitive to the sun. Indian coolie women blacken the surrounding of the eyes of their children from birth, and this practice is found beneficial by mountain climbers for mitigating the glare of snow. The black prevents the reflection of light from the skin of the nose, &c., into the eyes.' The parts of the hide of 'white' Indian cattle that are black are the lower parts, which do not get the direct rays of the sun. In 'white' men the eye sockets, scrotum, arm and knee pits retain their hair covering, but these parts are less hairy in cattle.

W. Lowe points out that all animals exposed to the sun's rays are more or less pigmented ; animals which are not pigmented live in the shade, white ants, worms, wood grubs, &c. The exposed parts of arctic animals, eyes, nose, and mouth, &c., are deeply pigmented. White fur effectually shades the skin and has been evolved to secure concealment. Pigment, while protecting from sun, is also evolved to secure concealment. It has become a racial character and persists unless causes arise which assail its stability. The black man has the highest

physical effectiveness in resisting the sun's rays, the white man the highest physical attractiveness. The change of environment means fresh adaptations and a new inheritance. As to the question of colonizing northern tropical Australia, Lowe[1] concludes that, apart from the question of tropical diseases, the white man in the black man's country degenerates mentally, physically, and morally, and his children are attenuated specimens of his race. The indolence of the white man and his dependence on black labour in the tropics are mainly the result of his physical depression caused by the heat, and his indulgence in spirits in his efforts to overcome his exhaustion. The white man only invades these lands to exploit them, make a competence, and leave them. 'A carpenter walks to his job with a Kaffir behind him carrying his bag. Bricklayers do nothing but put the bricks in place in a lordly fashion, the rest of the work is performed by blacks. Every artisan is essentially an overseer, and the blacks are his industrial valets.'[2] The white man's efficiency is thus undermined and the native trained to supplant him.

In the case of a Boer farm of 2,000 acres of excellent land—the house was a hovel consisting of one room divided into two by a dirty bag curtain—the Boer lads, ignorant and wholly illiterate, seldom completely undressed and hardly ever washed their bodies; nobody worked except the Kaffirs. The family loafed from dawn till eve, lounging or sitting on the step, smoking, drinking coffee, rarely indulging in a little conversation. Here, again, the question arises how much are such conditions of life due to climate and how much to want of education—they must very largely be due to the latter cause.

Lowe says any of us who have lain suffocating on the deck of a steamer as it passed through the Suez Canal, and have seen the native Arab children racing along the banks begging for 'backsheesh', must be compelled to admit that the Arab has acquired some immunity which the white man does not possess. 'The white man protected by all that science can give him; the Arab sublimely ignorant of the perils of light, heat, and a humid atmosphere.'

Against this view must be put the fact that the whites sitting on deck are over-clothed by custom and fashion, out of condition, not hardened by exercise, and overfed on foods of high specific dynamic energy. The English soldier fought successfully on the banks of the Suez Canal, in Palestine, Mesopotamia, East Africa, showing what hard training can effect. This the Arab children have from birth. But few British-born soldiers want to stop in these climates. Their dislike may be from the call of the upbringing, not from the blood. The man wants the environment he is used to and was brought up in.

It must be borne in mind that the rate of vital reactions is increased by temperature, and life of insects is much shorter in warm than in cool surroundings, and life of natives is shorter in the tropics, e. g. the savages of New Guinea; the women are old and worn out soon after twenty; all the hard work is put on them; so factory hands in Britain may have their lives shortened by their indoor occupation. They live with their bodies at a higher mean temperature than agricultural workers.

[1] *Proc. Roy. Soc. Victoria*, 1912, **24**, 353. [2] Pratt, *The Real South Africa*.

Lowe contends that field labour in the tropics can only be done by pigmented races, and that inter-breeding should take its course between whites and coloured races so as to produce a virile people suited to tropical climates. The skilled whites at Panama, he says, required and received all the protection that American ingenuity could provide. The great mass of unskilled labourers there were coloured men.

In the tropics there are economic reasons which prevent the offering of a remuneration sufficient to tempt whites to field labour. Where remuneration is sufficient, as in the sugar plantations of North Queensland, whites do the field work, and do it better and far more profitably than Kanakas. I am told big blondes may be found there defying the forebodings of Woodruff. Dr. M. Culpin, who lived for some years in northern Australia, tells me the whites there are fine big men, who work out of doors stripped to the waist, face the sun, and are inured to a shade temperature averaging 75° F. Gaining the full advantage of wind and keeping up their metabolism by hard exercise, they do well. It is the women who fade by living indoors, ageing at 25–30 and losing their fertility.

Mr. F. D. Green, of the Orient Line, tells me Scandinavians, Irish, and Lombardians make the best stokers of steamers in the tropics; coolies are only fairly good in comparison.

Freer concludes that the climate of the Philippines, surrounded by sea which modifies extremes of temperature, with a large proportion of cloud, is not by any means deleterious to the white man if he takes ordinary precautions, which are not as elaborate as those taken in a northern climate to keep out cold. The water lost in sweating must be made good. 'Many of the untoward effects attributed to the sun are probably due to the rapid loss of water from the system and could be avoided if the individual were in a position to drink enough.'

The direful effects of the Philippine climate, so vividly depicted by Woodruff, relate to the earlier days of American occupation. These were due chiefly to infections resulting from poor hygienic conditions unavoidable in the early campaigns. 'It does not seem that any effort is now made to spare officers or men from exposure to the sunlight, yet the morbidity and mortality continually decrease.'

Affections of the nervous system, including insanity, are among the diseases considered by Woodruff to be particularly likely to occur in the tropics as a result of excessive light stimulation. In the later years of occupation there are no noteworthy differences between the admissions of soldiers for these affections in the Philippines and in the United States. Whether men of dark complexion living ten to twenty years in the tropics stand it better than fair people is impossible to determine from lack of data.

W. P. Chamberlain has investigated blonde and brunette soldiers who have spent two years in the Philippines, and concludes that heat and humidity produce the distress felt by whites, and there is no need to invoke the actinic rays. Blondes are quite as well able as brunettes to stand a tropical climate. C. Crossland,[1] writing of the Red Sea climate, says excessive sweating, prickly heat, and consequent mental

[1] *Sudan Notes and Records*, 1919, **2**, 198.

irritation and weariness, can be avoided by suitable conditions of clothing and housing, see Chaps. VIII and IX.

The results obtained by the sanitary administration of Gorgas in Panama, in Cuba, and the Philippines, and during the war in the tropical climates where the British have fought, lead to the conclusion that the main cause of deterioration of health of the Caucasian race in the tropics is infection, and a vast improvement has followed action based on the parasitic theory.

'The basis of the largest proportion of illness and death in the tropics is bad sanitation and not climatic influences', say Castellani and Chalmers.[1]

'In cleansing Cuba', says Sir Harry Johnston, 'and in making the Panama Canal, the white American has learnt the secret of the tropics, of how to live under an equatorial sun and amid torrential rain, and yet, by exterminating or avoiding insect poisoners, to keep his health and vigour.'

'Africa and negroid Asia', he says, 'seem to have been the great centres for originating and maturing the worst maladies which have afflicted, arrested, or exterminated mankind and his domestic animals. From India came dengue fever, small-pox, bubonic plague, cholera, Asiatic relapsing fever, dysentery, typhus, syphilis, the "surra" cattle sickness. From Africa sleeping sickness, the nagana or tsetse sickness among cattle, malaria (as well as from Asia), Zambezian relapsing fever, the "yaws", and parasitic worm diseases, filaria, guinea-worm, hook-worm.'

The semi-civilized or savage negroes go about with bare feet, live filthily, ignore sanitation, and are careless about drinking-water.

The hook-worm is the cause of the negro's anaemia and laziness—reckless about sanitation he defecates anywhere. The outskirts of African towns are noisome to a degree. 'In Jamaica there are rotting accumulations of human ordure sufficient to infect all bare-footed Jamaica with hook-worm disease.'

'Hook-worm affected to a greater or less extent the whole rural population of India; in many parts as many as 90 per cent. were infested. This debilitating disease, which renders the sufferers unfit for hard work and an easy prey to other diseases, could be eradicated by the use of properly managed latrines. The guinea-worm, propagated by minute animalcules (cyclops) swallowed with drinking-water, a scourge of India, might be entirely avoided by straining drinking-water through a cloth, or by boiling it. Typhus fever, spread by lice, could be avoided by cleanliness. The germs of cholera, dysentery, and typhoid were derived from human excrement, and reached man through polluted drinking-water or food or milk contaminated by flies or dust. Bubonic plague was spread by the infected rat flea, and might be checked by keeping rats out of houses and grain stores' (McCarrison).

Leiper has shown that bilharziasis is caused by two different species of worms, the intermediate host being two different fresh-water snails, the bilharzia entering the human body through the skin or mucous membrane of the mouth. Irrigation in Egypt has in recent years greatly increased the snail and with it the incidence of the disease, and unrest is caused by the high mortality, especially of the

[1] *Manual of Tropical Medicine*, London, 1919.

children of the natives. Efforts are being made to solve the problem by allowing periodic drying of the snails' breeding-pools and by sterilizing water used for ablution.

There is no doubt that the health of Europeans in the tropics can be very greatly improved by very simple means of prevention of infectious and parasitic disease, and such have been carried out in many places. By the destruction of mosquitoes yellow fever has been almost expelled from America.

The sanitary work of Gorgas at Panama has become world famous. The climate at Panama is not very disagreeable, for while it is warm all the year round, its temperature is not so high as that of many United States localities during the summer, and its rainfall is also exceeded in other places. The perpetual moist warmth constitutes ideal breeding conditions for mosquitoes. The great measure of prevention is drainage. Surface drains are cleared every two or three weeks, and grass and brushwood cut within 200 yards round all residences. Oiling the puddles and the use of quinine are subsidiary measures.

The probable limit of flight of anopheles, the carrier of malaria, is 200 yards. Stegomyia (yellow fever carrier) is a poor flyer, and spends about a week in the larval state in water.

The death-rate in 1906 among negroes was 45 per 1,000, and in 1913 it was 5 per 1,000, while among whites in 1906 it was 11 per 1,000, and in 1913 2·5 per 1,000.

Labour was more remunerative, says Gorgas, in the tropics than in temperate zones, and when the inevitable trend thereto was in full swing it would be gratifying to sanitarians to reflect that this was possible through the efforts of members of the Anglo-Saxon race.

Samarai, in the south-east of Papua, with a humidity and temperature almost exactly that of Sierra Leone, has never been notorious as a particularly unhealthy place. The difference is probably due to dangerous endemic diseases at Sierra Leone; mosquitoes have been exterminated at Samarai.

How satisfactorily preventive measures act is illustrated by the following: in Macedonia in one camp (Greek), no preventive means taken, 100 per cent. of the men infected with malaria. In a second camp, where quinine was given, 45 per cent. infected. In a third, where measures against the mosquito only were used, 25 per cent. infected. In a fourth, where both measures were used, 6 per cent. of the men and 3 per cent. of the officers infected.[1]

Rigorous measures have been taken in the malarial districts of Japan. There is now not a district affected by malaria except a few villages of Formosa.[2]

Sir Harry Johnston says:[3]

In Jamaica 'the white birth-rate seems to be small, partly owing to the large proportion of bachelors among the whites and the tendency of the white women in the tropics to bear fewer children.

'Nevertheless, with our increasing mastery over tropical diseases, there is no reason why a white population of at least half a million should not grow up alongside a negroid race of four millions in

[1] Castellani, *Proc. Med. Conf. Red Cross Soc.*, Cannes, 1919, 132.
[2] Kabeshima, *ibid.*
[3] *The Negro in the New World*, London, 1910, 272.

a perfectly cultivated Jamaica, provided the whites were allotted land in the central, cool, mountainous part of the island.'

It is important to note the reservation, 'provided the whites were allotted land in the central, cool, mountainous part'.

Disease spread by impure water, flies, mosquitoes, and parasites having been eradicated and habits conformed to the situation, W. A. Osborne hopes the white man may arrive at permanent occupation of the tropics.

Nevertheless, the fact remains that the life is not a comfortable or ideal one—the white migrant and his wife cannot make a happy home in the tropics and bring up with ease and content a family of healthy children, as they can in a temperate climate. The customary furlough allowed to white officials in the tropics, and the fact that they send their children home to England to ensure their upbringing, shows the strain put upon them by the climate.

As to the question of evolution, Griffith Taylor thinks a race may be evolved in two or three generations suited to northern Australia.

There is in progress an intermingling of the subspecies of mankind which inevitably increases with the freedom of intercommunication, an intermingling which will finally raise up a race not only tolerant of, but virile and happy in, each climate. However custom and law try to put a stop to the entry of coloured races into a particular country and keep it a white man's land, e. g. Australia, this must fail, because the hybrids, apart from negro characteristics, become indistinguishable from those of pure white descent, and these hybrids will prove more tolerant and breed and people those places where the climate tells against the European. Howsoever the white man lynches the black for interfering with white women, he himself continues to use as his concubines negro and other coloured girls, and so raises up a vast army of hybrids.

Sir Harry Johnston says that in Brazil 'the Portuguese mixed eagerly with the Amerindians and raised up a great, proud, and warlike intermediary race of Manelucos. Then in the late seventeenth century they and the Manelucos began to mix maritally with the imported negresses.' There has thus grown up a huge empire of mulattos, of mixed Caucasian-Negro-Amerindian blood.

'The Indian blood once introduced tends to eat up the other blood and to reproduce itself again and again. The same influences of food, climate, air, forest, hills, and a thousand imperceptible influences which are at work on the new comer, cause any drop of Indian blood in his children to bring about the Indian type. The product of European and Indian is a fine type, handsome, well-built, and nice in character.'

There are 110 million whites in the New World. The negro, in spite of bad hygienic conditions and infective diseases, strong drink, the wearing of clothes, continues to multiply in the tropical parts of America, and numbers some 24 million (1908), including 10 million hybrids with whites. There are 21 million hybrids between white and Amerindian in the New World. In Jamaica there are (1908) 15,000 whites, 654,000 negroes, 157,000 negroids; so the white men are busy raising hybrids there, while the white women are relatively infertile.

Sir Harry Johnston says the Amerindian, descended from an early branch of the Mongolic mixed with the Proto-Caucasian, easily mixes with whites—the hybrids becoming as whites, handsome according to the white ideal, and the olive of the skin permeated with a rosy blush. The negro, on the other hand, transmits pigment and other characteristic traits. Amongst the negro children and students at Hampton and Tuskagee there are hybrids with dark eyes and skins, and red, blonde, or golden hair, and even some blue-eyed, fair-haired negroids retaining only the undulating curl of the hair and the pale olive complexion.

Sir Harry Johnston has made the most complete exposure of the terrible history of cruelty and inhumanity of the white man against the negro. At the same time he has evidenced the comparative greater gentleness and less sexual viciousness of the latter, and pointed out that lack of sanitary law and consequent infectious disease has sapped his strength and rendered him feckless in the past.

As to the effect of the Australian climate on the whites, I cite the opinion of an observant physician:

'The Australians', he writes, 'grow up quickly, come to maturity quickly, and arrive at old age before their ancestors did; they always appear to live at high pressure, and have very little reserve of energy; beauty fades earlier than in colder climates, a trip to Europe will often rejuvenate them for a long period. They have already acquired a quickening of sense of sight and hearing, greater growth of hair over the body, a soft voice when speaking, early arrival at puberty—characteristics resembling the aborigines.'

Dr. M. Culpin tells me the children in North Australia wear great sun hats. When they stand in school their naked feet wet the floor with sweat. Convention keeps them clothed, pride of race making the mothers preserve their whiteness. They should be stripped but for shorts or some gauzy shirt widely open at the neck and with short sleeves. They should tan in the sun, be in and out the water on the sea-coast, live free open-air lives. It is indoor conditions which make the women fade, age, and become barren. If white children were brought up in the tropics naked but for a loin-cloth in the open, they would grow up strong and healthy, but tanned all over. It is convention and caste that tell against comfort and health.

CHAPTER IV

Emission, Absorption, and Reflection of Light by White and Black Skin.

'By the law of radiation', says Woodruff,[1] 'skin pigment is of enormous value in helping the body to radiate surplus heat if the environment is cooler than the body. It is a common observation in the tropics that dark races are quite comfortable at room temperatures which cause white men to drip with perspiration. On the other hand, dark pigment by the law of absorption is a disadvantage when the environment is hotter than the body, for the dark surface absorbs heat to a fatal degree.'

According to Woodruff, then, it is the higher emissive power of the black skin for radiant heat which adjusts the negro to the hot humid shade of the tropical forest, to conditions intolerable to the white, while the higher heat-absorbing power of black puts him at a disadvantage in atmospheres hotter than body temperature.

Richet, too, says a white rabbit loses only 75 per cent. of the heat lost by a black. Lefèvre also assumes that the skin of lizards radiates less heat when white than when dark.

Now the classical experiments of John Leslie,[2] with a cube filled with boiling water, showed that while there was an enormous difference between the emission of radiant heat from a polished metal surface and from lampblack, there was none between the emission from a surface of lampblack and that from a surface of white paper.

Leslie covered the sides of his polished metal box with (1) lampblack, (2) writing-paper, (3) glass. Taking the black as emitting a radiant effect of 100, the white paper emitted 98, the glass 90, the bright polished side 12. When the white paper was rubbed with white chalk it emitted 90. Some white soft glossy papers even surpass lampblack. A thin wet film of gelatine raised the emission of the polished side to 38, and a film four times thicker to 54. The emission of a dry isinglass film was 80, sealing-wax 93, rosin 96, ice 85, china ink with gum in it 88. Roughing the polished surface with parallel striae more than doubled the radiating power of that surface.

Investigating the power of reflecting radiant heat, and taking that of the polished brass surface as 100, polished silver gave 90, steel 70, lead 60, Indian ink 13, glass 10, oil 5, lampblack 0. Furrowing the polished surface lowered the measured reflection to $\frac{1}{10}$th of its former value, dispersion taking the place of reflection. The skin is a furrowed surface comparable to paint, paper, isinglass, and to wet isinglass when wet with sweat.

[1] *Medical Ethnology*, New York, 1916.

[2] *An Experimental Enquiry into the Nature and Cause of Heat*, London, 1804.

To measure the effect of light on the skin, I used a thermopile which Sir James Dewar kindly lent me. This thermopile is enclosed in a jacket through which water at a constant temperature circulates. In the jacket and opposite to each other are two small conical openings closed by corks. On opening one or other of these, the skin, or other surface, can be brought to radiate upon one or other surface of the thermopile. Meanwhile the other surface of the pile is kept at the temperature of the jacket, which shields the thermopile from all other sources of radiant heat. A uni-pivot galvanometer, made by Paul, of 10·8 ohms resistance, is used with the thermopile. The deflections on the scale are not large, but can be read accurately with a lens. The thermopile is placed in such a position that convection currents coming from the skin do not influence it.

The intensity of the radiation at a point, due to a given source, is inversely proportional to the square of the distance of the point from the source. As the surface of the skin is withdrawn from the thermopile, the intensity of the radiation received by it from each point of the skin decreases, but the area from which the radiation is received increases. The total amount received depends on the product of these two factors, and this remains constant so long as the cone projected from the conical orifice in the jacket falls upon the skin-surface, so that the thermopile receives rays from no other source. The distance, then, of the skin from the thermopile is of no importance so long as this condition is secured.

The emissive power at 37° C. of glass, gelatine, water, or skin-surface was found to be about the same. Thus

Indoors; dull, diffuse daylight.
Temperature of water in jacket, 20° C.
„ air, 21·5–22° C.

Surface	Deflection in degrees of scale	Surface temperature
Extensor surface of forearm just bared	4·0	33·5° C.
Palm	4·5–5	35
Cheek	5	35
Open mouth	5·5	36
Balls of fingers	4·5	35
Armpit	5	—
Sole of foot just bared	4·5	—
Glass containing water at 37° C.	5·5	36

The radiation emitted from the warmest parts of the body is, then, the same as that emitted from the surface of a glass of water at body temperature.

Out of doors; fine August day, sheltered.
Water jacket, 17° C.; air, 16° C.

Surface	Deflection	Surface temperature
Palm in sun	16	37° C.
Palm in shade	5·5	34

Out of doors; in moonlight, clear, cool, calm.

Palm	3·5	—

Out of doors ; overcast, cold wind.

	Deflection	Surface temperature
Palm in wind	1·0	21·8° C.
,, shelter	3·0	23·0
Cheek in wind	3·5	25·0
,, shelter	5·0	27·0
Back of hand in wind	0·5	19·7
,, shelter	3·0	22·6

The wind cools the surface film from which the radiant heat is given off more than is shown by the surface mercury thermometer, which, by shielding from the wind, appears to give too high a temperature.

If a thermo-junction is used in still air the readings of surface temperature closely approximate to those of the mercurial thermometer. In variable wind the needle of the galvanometer is never still, and the thermo-junction readings at any moment may vary widely from the average given by the thermometer. The monotony of effect on the nerve-endings of still air compared with outdoor moving air is thus demonstrated.

Leslie cube filled with boiling water.
Water-jacket, 10° C.; air, 16° C.

Surface	Deflection in degrees of scale
Black, rather glossy . . .	21
White paint	21
Polished but rather dull tin . .	2

Leslie cube at room temperature.
Water-jacket, 10° C.; air, 16·5° C.

Reflection of parallel beam of light from arc lamp, the angle of reflection being kept the same.

Black	4
White paint	9
Polished tin	42

Similarly in the shade the skins of white and black subjects at the same surface temperature give the same deflection.

In the sun the white skin gives a deflection due to diffusive reflection almost double that of dark negro skin.

The forearm of a dark negro from north Africa illuminated with the arc lamp, a water-screen being interposed, gave a thermopile deflection of +2·5 over the shade value with a surface temperature of 36° C., while the figures for the white forearm were +4·5 and 35° C.

With the naked arc the surface of the negro's forearm was heated up rapidly to over 45° C. It was not possible to observe the difference of reflection between black and white skin owing to the rapidly increasing heating effect on both.

In the October sun (calm day) the negro's arm gave a deflection of +2 while the white arm gave +5 above the shade value. Examined with the spectroscope and compared with that from white skin, the light reflected from the negro's skin gave a dimmer red, the green, blue, smoky, and indefinite, and great shortening of the blue, violet. A melanin screen cuts off all but the red rays, these also if in a thick layer. The following figures sent me by H. E. Roaf show that there

is no average difference between the surface skin temperatures of Europeans and Egyptians.

Contrast of skin temperatures. Observation made in Egypt (Roaf).

Date	Time	European		Egyptian	
		Shade	Sun	Shade	Sun
3. 12. 14	14.00	31.5° C.	36.4° C.	31.0° C.	35.5° C.
4. 12. 14	14.30	33.0	37.7	32.5	35.5
5. 12. 14	14.00	33.0	39.8	33.5	39.5
10. 12. 14	14.00	32.4	40.0	31.5	38.5
30. 3. 15	13.40	34.0	39.3	34.0	39.0
6. 4. 15	13.45	33.5	38.2	34.3	39.0
12. 4. 15	13.45	33.0	39.5	35.5	39.8
13. 4. 15	13.30	35.8	39.4	35.8	38.7
17. 4. 15	13.30	33.5	37.8	33.9	35.9
18. 4. 15	13.45	33.3	39.5	34.2	39.5
26. 5. 15	12.30	34.5	39.3	37.0	40.2
27. 5. 15	12.30	36.2	39.3	37.3	41.0
6. 6. 15	12.45	35.0	39.2	35.3	40.0
22. 6. 15	13 30	34.5	39.5	35.4	40.2
23. 6. 15	13.30	34.8	39.5	36.6	41.0
11. 7. 15	12.45	35.6	40.2	36.5	40.5
3. 8. 15	13.30	33.3	39.0	34.8	39.8

European average in sun 39·0° C.
Egyptian „ „ 39·1° C.

As sweating lowers the temperature dry white and black skin surface require to be compared.

Melanin was prepared from ox eyes. The irides and choroidal fringes being extracted with boiling alcohol, ether, water, and digested with trypsin, the nuclein was removed by boiling with alkali. The residue was then dried and ground into a fine powder. A film was made of this about as brown as negro skin, and the thermopile deflection and surface temperature were observed on exposure to the October sun:

Surface	Deflection in sunlight	Surface temperature
White paper	16 divisions of scale	19° C.
Melanin film	7 „ „	21° C.

The black pigment absorbs more, becomes hotter, and reflects less. White and sooted hands were then compared:

Surface	Deflection in sunlight	Surface temperature
White hand	10 divisions of scale	30° C.
Ditto, blackened with soot	4 „ „	32° C.

The black skin of a bulldog's lips were next compared with the white skin of the hand, and the following surface temperatures seemed at first to confirm Woodruff's view that the black skin emits more heat in the shade than the white skin.

September mid-day, sun, warm:

	Surface temperature		
	Out of doors in sun	Indoors in shade	Difference
Bulldog's black upper lip	41° C.	29° C.	12° C.
White hand	37.5	33.5	4

Afternoon, weaker sun:

	Surface temperature		
	Out of doors in sun	Indoors in shade	Difference
Bulldog's black nose	37° C.	25° C.	7° C.
White hand	36·5	33·5	3

But against this is the fact that the dog's lip and nose are naturally damp, and so cooler surfaces in the shade, while they become dry and warmer than the white hand in the sun. That this view was correct was confirmed by comparing the reading of the black inner hairless surface of the dog's ear with the white ear.

Afternoon, weaker sun:

	Surface temperature		Difference
	In sun	In shade	
Bulldog's ear	37° C.	33° C.	4° C.
White ear	36	34·5	1·5

The *dry* ear temperatures in the shade were much nearer together.

I then secured readings from naked piebald piglings and piebald furry animals. Two black-and-white piglings were examined in the shade and in the October sun with cool breeze. The surface temperature of the black patches in the sun were 36°–37° C., the white patches 34°–35° C. The black fur of a piebald rat was 2°–3° C. warmer than the white fur in the February sun indoors. In the shade all parts of pig or rat were at the same temperature. So too with the black and white fur of a bulldog, cat, guinea-pig, the white hand, and one sooted black.

In the sun the temperature of the black parts is higher, in the shade the temperatures of black and white are equal.

A piece of black fur and a piece of white fur were laid on a window sill indoors in the February sunlight, and the bulb of a thermometer on each. The black became 6° C. hotter than the white. In the March sun it was 12° hotter.

The next figures show how much blackening raises the surface temperature in the sun. The increased deflection in the sun is not due to increased surface temperature but to diffusive reflection.

September day, warm sun, out of doors. Water-jacket, 17·2° C.; air, 18·3° C.:

Surface	Increased deflection on exposure to sun	Surface temperature In sun	Difference of, between black and white
White blotting paper	9° C.	25° C.	4° C.
Ditto, blackened with soot	1·5	29	
Bright tin lid	off the scale	28·5	3
Ditto, blackened with soot	1	31·5	
Glass mirror	off the scale	26	14
Ditto, blackened with soot	0·5	rising over 40	
Palm of white hand	4	32·5	2·5
Ditto, blackened with soot	1	35	
Black fur of bulldog	1	42	

A ship's crew stripped to the waist and coaling in the sun must have to sweat more owing to the blackening of their skin.

A blackened palm feels in the sun hotter and drier than the white palm; this was particularly the case when the circulation was stopped

by a tourniquet. On letting the blood in, relief to the hot dry sensation was felt. There is a grateful feeling of moisture and coolness, the surface temperature drops a little, while the deflection does not alter.

Out of doors in sun, gentle breeze. Water-jacket 17° C.; air, 18·5° C.:

		Deflection	Surface temperature
Palm circulation	in sun	12	32·2° C.
Stopped by tourniquet	in shade	5	31·5

In the next case the forearm was made bloodless in the shade, exposed to the sun, and then the blood let in:

Forearm bloodless	in sun	10	32·0° C.
„ blood let in	in „	12	35·0

The surface of the skin cooled by water gives a less deflection and surface temperature, warmed at a fire a higher deflection and surface temperature.

September day, overcast after rain. Indoors: Air, 18·3° C.; water, 15·5° C.:

	Deflection of galvanometer	Surface temperature
Palm	4	27·5° C.
Palm after putting in cold water	3	25·5
Palm after warming at fire	5	30

On cooling the palm with a little carbonic acid snow rotated round in it, or by a metal rod cooled in liquid air, the cuticle remains cold, and feels cold for several minutes. The tongue can sense the cold patch.

Palm cooled by metal rod dipped in liquid air:

Time	Deflection	Surface temperature
50 sec. later	—	24° C.
70 „ „	2·5	26
115 „ „	3·0	27·5
150 „ „	3·5	28·5
The other palm	4·5–5	32

Similarly after warming the palm at a fire till the surface temperature is well over 40° C., it takes some time for the surface to cool down. The last stage of equalization is of course the slowest.

These last observations demonstrate how bad a conductor the cuticle is, how it protects deeper layers, and how thermal sensation arises from differences of surface and deep temperatures. The after-sensation of cold felt on removing a penny which has been pressed against the forehead, is not a positive after-image, but is due to the cuticle remaining cold.

The influence of pigment on temperature of cold-blooded animals. Apart from protective coloration and the absorption of lethal rays, pigment is used according to Lefèvre for modifying body temperature by certain cold-blooded animals which love to bask in the sun. The lizard, Uromastix, and the monitor, Varanus, exposing themselves to the sun, warm up to 39° C. and develop a polypnoea with open mouth, making 20°–36° shallow respirations per minute. The

polypnoea is first excited by the heat of the sun on the head, continuing, however, if the body temperature rise above 39° C. when the rays are cut off from the head. The polypnoea is superadded on to the respiratory function, and does not disturb or interfere with this. As much as 12 grm. of water per kg. per hour may be evaporated thereby, approximating to the sweat lost by a man in a warm atmosphere. The lizards according to Lefèvre expand their pigment cells and become sombre in colour and so favour heat absorption, becoming clear when too warm. The monitors reach 6 ft. long and are used to assist burglars, the lizard with a rope round it being made to scale the wall, and by its weight and holding power helping the burglar over! Made warm by the absorbed sun energy they become for most of the 24 hours as active as the hot-blooded animals, cooling down and becoming sluggish during the night, again seeking the sunlight to warm in after sunrise.

Lefèvre suggests that the lizards become light coloured at night and in the shade, so as to lessen the emissive power of their skin and preserve as far as possible an optimum temperature; this hypothesis was proved untenable on p. 69.

In fishes, amphibia, and reptilia, changes in colour pattern are due to the reciprocal interplay of dark pigment-bearing cells (melanophores) and cells bearing various other pigments, many of them with metallic lustre (silvery xantholeucophores). In diffuse daylight, on an indifferent background, and still more in cool darkness, the epidermal melanophores of the tadpole expand and the corial xantholeucophores contract. The simultaneous action of warmth (33°–35° C.) and sunlight has the opposite effect.[1]

It seems probable that the evolution of the warm-blooded animal has taken place through animals which, warmed by the sun and succeeding in the struggle for existence through the increased rapidity of action produced by warmth, evolved mechanisms for maintaining the heat; mechanisms which were first directed to retaining the sun heat and lessening heat-loss at night, and finally supplemented by the evolution of a regulative mechanism of heat production. The fact that the body temperature is lowest in the early morning may be associated with the far-off days when the primitive type was daily warmed up, like Uromastix and Varanus, by the sun and cooled down to the lowest point before sunrise. The thermo-regulating animal may still be slightly warmed in the sun and cool in the shade. In the duck-billed Platypus and Echidna, C. J. Martin [2] detected a partly evolved heat-regulating mechanism.

[1] P. E. Smith, *Sc. Pr. Soc. Biolog.*, 1919, **16**, 1484.
[2] *Tr. Roy. Soc.*, 1902, **195 B**, 1.

CHAPTER V

The Influence of Sunlight on Transpiration of Wet Black and White Surfaces.

To investigate further the influence of sunlight on black and white surfaces, I blackened a kata-thermometer by dipping it in a mixture of absolute alcohol, dextrin, and lampblack, and whitened another by dipping it in a mixture of absolute alcohol, dextrin, and zinc white. The katas were dried and dipped again and dried till a good uniform coat was obtained on each.

I then measured the rate of cooling in the sun and in diffuse daylight. Indoors in front of a window in the weak November sun, the black cooled from 100°–95° in 73 sec., the white in 41 sec. In diffuse daylight they cooled in the same time. On another day the black cooled in the winter sun indoors in 150 sec., the white in 85 sec. Out of doors in the sun and a cold wind, the black cooled just a little the slower; the wind was effectual in almost equalizing the cooling rates. Nevertheless the radiant energy of the sun falling on the surface of the skin, in spite of the cooling wind, gratefully affected the nerve endings; indoors of course the sun felt quite warm.

The smooth surface of the black kata is cooled by the wind, while a piece of black fur both absorbs the sun energy and, entangling the air, is not cooled by the wind to anything like the same degree.

Taking a white paper with a black letter stencilled on it, I put it in the parallel beam of an arc lamp and explored the surface temperature with a thermo-electric junction. The black gave a deflection of 10 degrees of the galvanometer scale more than the white. On wetting the paper the black still gave a greater deflection, nearly 5 degrees greater than the white, and the water evaporated from it more quickly, so that it dried first. The wet black surface therefore absorbed the light rays, became hotter and accelerated evaporation by raising the vapour tension.

To demonstrate further the influence of sunlight on the evaporation of water from white and black damp surfaces, I took two small cylindrical clay filters and stained one blackish—about the black of a negro. These pots I filled with water, and corked with rubber corks, in each of which was inserted a length of tubing. The tubes were of the same bore and filled with water up to a mark. The filters were placed in the horizontal position on a window-sill indoors in the February sunlight.

The rate of evaporation was measured in cms. by the change in the position of the meniscus in a given time. The capacity of filters and tubes and the surface of filters were measured, and the evaporation calculated per sq. cm. per sec. The temperature of the water inside the filters was taken before and after. The experiment was repeated in the shade.

The sunlight was proved to accelerate evaporation by the increased absorption of rays and their conversion into heat by the black surface.

Vol. evaporated by black filter in shade = 0·72 c.c. in 100 min.
" " " white " " " = 0·64 c.c. "
" " " black " in sun = 2·36 c.c. "
" " " white " " " = 1·74 c.c. "

Surface area of black filter = 63·5 sq. cm.
" " " white filter = 59·1 sq. cm.

Volume of water evaporated in shade in 100 min.: black 0·72 c.c., white 0·64 c.c.

Taking latent heat of evaporation as 588·5 cal., loss of heat per sq. cm. per sec. by evaporation in shade is

$$\text{Black} = \frac{0{\cdot}72 \times 588{\cdot}5}{63{\cdot}5 \times 100 \times 60} = 1{\cdot}11 \text{ millicalories}$$

$$\text{White} = \frac{0{\cdot}64 \times 588{\cdot}5}{59{\cdot}1 \times 100 \times 60} = 1{\cdot}06 \text{ millicalories}$$

Difference 4·7 per cent.

Volume of water evaporated in sun: black 2·36, white 1·74.

Half the filters are in shade; deduct half above shade value, then

Volume of water evaporated by sunny half is

Black $2{\cdot}36 - 0{\cdot}36 = 2{\cdot}0$ c.c.
White $1{\cdot}74 - 0{\cdot}32 = 1{\cdot}42$ c.c.

Loss of heat per sq. cm. per sec. by evaporation in sun is

$$\text{Black } \frac{2{\cdot}0 \times 588{\cdot}5}{31{\cdot}7 \times 100 \times 60} = 6{\cdot}10 \text{ millicalories}$$

$$\text{White } \frac{1{\cdot}42 \times 588{\cdot}5}{29{\cdot}5 \times 100 \times 60} = 4{\cdot}7 \text{ millicalories.}$$

Difference 30 per cent.

The water in the black was at 16° C. and in the white filter at 14° C. at the end of the observation.

Another way of demonstrating the same fact was this. I took two equal lengths of white cord and dyed one black. The two cords were equally weighted at one end and then soaked in water. They were then suspended from the middle bar of a window frame in the February sunshine, so that the weighted ends were at the same horizontal mark on the lower bar of the window frame. The black cord dried quickest and extended its length, first showing the accelerated rate of evaporation produced by the absorption of rays by the black. In the shade at dusk the two cords extended equally and together. Bright skyshine, apart from direct sunlight, made a difference. I also took two equal lengths of a broad cotton shoe lace, one black, the other white, and suspended them in front of the window, one end of each dipping into a 10 c.c. measure glass of water. The evaporation from each was measured first in the shade and then in the sunlight. Here again the black evaporated far more; this method affords the easiest and most striking demonstration.

Rate of evaporation from black and white broad cotton shoe laces (made of the same material), when suspended in a cold greenhouse in front of the glass:

White	Black	February 27 and 28
2·5 c.c.	2·2 c.c.	During cold night.
0·6	1·3	In sun early, 2 hours.
1·5	2·5	Midday sun, 2 hours.
1·0	0·9	Bright clouds, 3 hours.
1·6	1·2	Dull, cloudy afternoon, 4 hours.
2·6	3·6	Midday sun, 3 hours.

A pair of broad white cotton shoe laces, one dyed black:

White	Black	March 1
2·0	2·0	During cold night.
0·5	1·4	Early sun, 2 hours.
1·4	1·4	Clear sky, sun off.
		February 27
0·7	1·2	Sun and cloud, 1 hour.
1·4	2·0	Sun and mostly cloud, 2 hours.
		March 2
3·6	3·6	During frosty, clear, windy night. Frozen laces.

I repeated the experiments with like results, using a piece of white tape and a piece of the same tape dyed with melanin separated from ox eyes.

In the case of black skin the sunlight absorbed and converted into heat by the pigment must similarly increase the transpiration of water from the capillaries in the papillae of the cutis and the evaporation from the skin surface. At the same time the heat, stimulating the nerve plexus in the epidermis which interlaces the pigment cells, will provoke vaso-dilatation and sweating, probably by an 'axon reflex', acting through the posterior root fibres of the spinal nerves. The pigment, on this hypothesis, acts as an activator to the mechanism for cooling the body by evaporation. The water poured out on the skin surface makes it glisten and act as a polished surface reflecting sunlight. The evaporating water-vapour surrounds the body with a film of mist which both absorbs and scatters the sun rays and mitigates their fierce assault.

To demonstrate this effect I took two kata-thermometers and covered one with a coat of thin white paper, the other with a similar coat of black paper. The paper was drawn up over each kata and tied round the stem. Some air was unavoidably enclosed between it and the kata. The kata with the black paper warmed up quicker than the one with the white paper in front of the gas fire. The katas were suspended in an opening in the side of a box so that the radiant heat acted equally on each, while draught was avoided. On wetting the paper covers, the kata with the black cover warmed up slower, because the rays, absorbed by the black paper and converted into heat on the surface of the paper, increased evaporation there and cut off the heat from reaching the kata beneath. There is no difference between white and black muslin covers, because these are too equally penetrated by the rays.

1.2.18. Heating of two katas with the same factor, clothed, one in black, the other in white, paper cover:

Dry	Time of heating 95°–100° F.	Temperature of air Initial	Final
Black	180 sec.	13° C.	13·5° C.
White	175	15	14·5
Black	150	14·5	14·8
White	157	15	15·5
Black	148	15·5	15·5
White	155	15·8	16·0
Black	145	16·5	16·2
White	155	16·5	16·5
Black	141	16·5	16·8
Wet			
Black	85	32·0	32·5
White	77	33	34
Black	85	34	34·5
White	80	34·5	35
Black	93	34·8	35
White	80	35·8	35·8
Black	91	36	36
White	87	37	37
Black	100	37	37
Dry (no coat)			
506	30	37	37
504	31	37	37
506	30	37·2	37·2
504	30	37·5	37·5
506	30	37·5	37·5

The dry paper coat cut down the cooling power of the dry kata exposed to the gas fire to one-fifth of what it was when naked, showing the enormous effect of clothes in absorbing heat from a source of radiant energy and so impeding loss of body heat in still air.

The following table gives the effect of a dry over-jacket of crepon in cutting down the cooling power exerted on the wet kata in Egypt (Capt. H. E. Roaf). Such a jacket about halves the cooling power, whether it be white or black. The wet kata with a finger-stall of black crepon appears to cool more quickly in the sun than in the shade. Not so with a finger-stall of white crepon:

	Kata covered by wet finger-stall of		Dry white crepon over-jacket		Dry black crepon over-jacket	
			Shade	Sun	Shade	Sun
17. 10. 17	Black crepon		10·0	7·55	8·7	6·6
19. 10. 17	,,		10·8	7·7	10·0	9·6, 8·4
20. 10. 17	,,		12·3, 12·0	7·75	8·7, 9·3	8·0
	Black crepon Shade	Sun				
3. 12. 17	25·2	18·9	12·3	10·2	12·3	8·8
12. 12. 17	Black crepon		13·5	9·8	11·7	8·5
	White ,,		12·3	10·2	11·7	9·5
	Black crepon Shade	Sun				
15. 12. 17	29·3	27·8	12·9	11·7	14·3	11·7
	White crepon Shade	Sun				
15. 12. 17	26·4	27·8	15·1	15·1	12·0	9·8

The following kata-thermometer readings, taken in Egypt by Capt. Roaf, show the relatively high wet kata cooling power which pertains there in the sun.

Date	Time	Dry	Wet
30. 11. 14	14·30	4·42	27·8
		3·63	20·8
3. 12. 14	14·00	8·75	31·2
			29·4
4. 12. 14	14·30	4·42	25·0
16. 3. 15	14·00	3·86	20·0
20. 3. 15	13·40	2·86	22·8
30. 3. 15	13·45	—	16·7
12. 4. 15	13·45	—	19·3
13. 4. 15	13·30	—	26·3
18. 4. 15	13·45	2·03	21·8

CHAPTER VI

Sunlight in Tropical and Temperate Climates.

Of the theoretically possible sunshine it has been computed that

Santa Fé	receives	76 per cent.
Denver	,,	69 ,,
New York and Chicago	,,	56–57 per cent.
Manila	,,	51 per cent.
English Channel	,,	43 ,,
England S.E. and S.W.	,,	38 ,,
England Mid. and N.	,,	31 ,,
Scotland W. and E.	,,	30 ,,
Scotland N.	,,	26 ,,

Aqueous vapour has an absorbing power for radiant heat 72 times greater than dry air (Tyndall). It is estimated that 40 to 50 per cent. of the sunlight is absorbed by a clear atmosphere, 30 per cent. with the sun at the zenith, 75 per cent. with the sun near the horizon. The insolation at high altitudes may be intense, and the sky look black rather than blue owing to the less scattering of light.

Atmospheric transparency and diathermancy are then the important factors in regard to solar insolation in different climates. On perfectly clear days these are much the same at widely different places. For example, Ångström [1] found these pyrheliometer values in Teneriffe at noon, 1·38 at Grumar (360 metres altitude), and 1·618 at Alta Vista (3·352 metres altitude). At Cape Horn (latitude 55° 31′ S.) the maximum intensity of solar radiation is given as 1·47, at Washington (latitude 38·5° 4′ N.) 1·44.[2]

The maximum intensity of solar radiation is surprisingly uniform throughout the months, 'the December minimum being only 8 per cent. less than the April maximum'. A year such as 1903 may give an average of 19 per cent. less than the quinquennial mean. In this year the diminution was widespread.[3]

The actual number of hours of insolation per year on the earth's surface, were the sky always clear, is greatest at the equator and diminishes towards the poles, the ratio between 0° and 45° latitude being 1·83 to 1·34.

The radiation from the earth in middle latitudes is about 0·1 of the insolation at midday. It acts during the entire 24 hours, whereas the sun rises and sets. In northern climates the hours of insolation during the short days are so few and the hours of radiation so many during the night, that the surface of the earth cools at certain times of the year, making one of the factors which causes a winter season.

[1] *Astrophys. Journ.*, 1899, **9**, 342.

[2] H. H. Kimball, *Bull. U.S. Mt. Weath. Obs.*, 1910, **3**, 100; P. Freer, *Philippine J. Sc.*, 1912, B. **7**, 1.

[3] There is some evidence of cyclical periods of colder weather and poorer harvests.

How hot the surface of the earth may become, exposed to tropical sun, is shown by the records taken at Chinchochro, Loango coast, near the equator. Here the surface of the earth exposed to the sun gave temperatures generally over 75°, often 80°, and one time nearly 85° C.[1]

The temperature of the sand near Cairo in the sun reaches 145° to 165° F. Steel tools lying on the ground are too hot to handle without gloves.

At Davos, Switzerland, altitude 1,559 metres, the average of the maximum black-bulb (*in vacuo*) readings for three years was 53·8° C. with a maximum of 67° in 1910. The maximum for Manila was 56°, 1910–11, for Helwan, Egypt, 70·8°, for Alexandria 57°, for Cairo 79·5°, for Aswan 81°, where the atmosphere is exceptionally clear and there is much reflected light from the desert, for Leh in Thibet, altitude 3,517 metres, 101·7° with a shade temperature of 23·9°.

The ultra-violet extends no further in the spectrum of tropical sunlight than in that of temperate climates.

The decomposition of a solution of uranyl acetate and oxalic acid has been used as an indicator of the total photo-chemical effect of the sunlight. It is affected by rays extending from 550 $\mu\mu$ to 291 $\mu\mu$, the limit of the sun's spectrum. At temperatures of 25° to 30° C. the temperature coefficient may be neglected, but in cold climates it must be taken into account, for the decomposition at 2° C. is only 60 per cent. of that at 30° C.

The sunlight is certainly not more oppressive in Honolulu than in the Philippines ; the temperature is much lower there, the average at the time of observation 21°–22·6° against 30°–35° in Manila, but the average solar photo-chemical effect is higher at Honolulu than at Manila. It is the amount of haze and cloud that makes the difference in comparing such places as Manila (latitude 14° 36′ N.), Honolulu (latitude 21° 18′ N.), Kuala Lumpur (latitude 3° 10′ N.), Khartoum (latitude 15° 36′ N.), Washington (latitude 38° 59′ N.).

A clear sky and dry air give the greatest insolation. As on clear days, when the sun is at the same angle, the rays are everywhere much alike in intensity, the biological effect of a tropical climate cannot be attributed to action of the chemical rays.

The infra-red rays are the more numerous when there is great humidity of the atmosphere, there being a great lessening of the blue-violet rays.

Humidity and stillness of the atmosphere and infra-red heat rays together give conditions which are badly borne.

In many localities the thermometer in summer often is much higher than it is in the Tropics, yet the heat by no means produces the same effect. It is high humidity in the Tropics and lack of wind which are factors of great influence. In Manila, for example, fresh winds from May to August make conditions much more tolerable than in certain parts of the Chinese coast or even on the Atlantic seaboard in places further to the north than Manila. The majority of people in the tropics are on the coastal or intermontane plains where high air temperature and humidity are continuous.

[1] Hann, *Handbuch der Klimatologie*, Stuttgart. 1910, **2**, 23.

CHAPTER VII

Black Fur, Cheek, and Clothes Surface Temperatures.

For measuring radiation of the sun, black-bulb thermometers in a glass evacuated jacket have been used. The readings vary with the thickness and diathermancy and, perhaps, size of the enclosing bulb, the perfection of the vacuum, the absorbing power of the black surface of the thermometer bulb, and the size of the black bulb.

The readings of instruments of the same pattern may be 15° F. apart. The position of the thermometer in regard to surfaces from which sunlight is reflected makes a great difference. Thus J. Aitken [1] found a sheet of white note-paper, placed near, and facing the sun, on a May day affected the readings as follows:

Temp. in screen	Black bulb	Black bulb, white paper	Difference
72° F.	125° F.	162° F.	37° F.

The black bulb gives high readings in winter out of all proportion to the sun's heating effect on the earth, which is small owing to the low angle at which the rays strike the surface of the earth.

The black bulb being *in vacuo* is not affected by the wind, and thus may give high readings when the feelings are chilly on a sunny day with strong wind.

Night radiation temperatures are usually taken by placing thermometers on grass. The grass loses heat by radiation into space, and the fall in temperature is determined by the amount of radiation on the one hand, and by the rate of supply of heat on the other from the ground and air. The supply of heat from the air depends on wind, so that on calm nights the grass temperature falls much below that of the air in the screen. Hollows and areas protected by trees, hedges, &c., give lower readings than exposed situations. The supply of heat from the ground depends on the conducting power of the soil and turf. Thick turf acts as blanket. Bare patches give the higher readings on clear nights, e. g. the turf 10° and the bare patch 5·5° to 7° below the screen thermometer.

The ordinary Stevenson screen gives too high temperatures when there is sunshine, it requires a double top with air space between, and the bottom to be closed with wood to prevent radiation entering below.

Aitken used a black hollow globe of metal or glass with the bulbs of maximum and minimum thermometers placed at the centre of the ball, or a flat shallow box packed with cotton wool, the upper surface black and with maximum and minimum thermometers in contact with the under side of the upper surface. These instruments are affected by the wind. Comparing the temperatures of the black ball with those of the black bulb *in vacuo* when there was much wind in July and August, the relation was 0·4 to 1, with little wind 0·66 to 1.

[1] *Journ. Scot. Meteor. Soc.*, 1911, **15**, 295.

The surface temperatures of black fur and the cheek are, I find, valuable indicators of the effects of sun and wind on the naked and clothed parts of the body.

On contrasting black fur and a black metallic surface exposed to the sun and wind, one can see the enormous power of fur to conserve the heat which is absorbed and prevent loss by convection.

To a piece of blackened platinum foil 0·25 sq. cm. in area, a junction of copper and eureka wires was soldered. Another junction was inserted in a piece of black fur. The circuit was so arranged that the temperature of either the foil or fur might be recorded. The control junction was kept in water within a vacuum flask. The board holding the foil and fur was supported vertically in front of a gas fire turned low and at a distance of about 70 cm.

After allowing time for conditions to become uniform alternate readings were taken.

The following results were obtained:

Fur	111	Divisions of galvanometer scale	= 43° C.
Foil	108	,, ,,	= 42·4
Fur	114	,, ,,	= 43·8
Foil	108	,, ,,	= 42·4

The effect of screening by glass was then tried:

	Fully exposed	Screened by glass lantern slide
Foil	96 = 39·4° C.	77 = 34·6° C.
Fur	108 = 42·4	84 = 36·4 (still slightly moving)
Foil		76 = 34·4
Fur		82 = 35·8

A wooden screen was next tried and the time taken for the galvanometer needle to rise (or fall) from 100–90 (= 40·4°–37·8° C.) when this was removed or introduced between the fire and the thermo-junctions:

Fur, fell in 37 sec.
Fur, rose in 67 sec.
Foil, fell in 4·5 sec.
Foil, rose in 12·5 sec.
} The window in the side of the room opposite the fire was open.

Foil, fell in 4 sec.
Foil, rose in 11 sec.
Fur, fell in 35 sec.
Fur, rose in 60 sec.
} The window was shut.

The air temperature was 26·5° C. near the thermo-junctions.

On introducing the screen in the case of the fur the galvanometer needle took 10 sec. to move through 1 galvanometer division (= 0·2° approx.) from its initial position.

With the foil in the circuit the slightest air current or variation in the fire caused movement of the needle.

Observations were next taken in a garden, exposed to sun, cloudless sky, fresh breeze.

Wind velocity, as given by kata readings taken in shade close to apparatus, the temperature being 14°–15° C., was 1·2, 1·6, 0·6, 1·4, 1·4 metres per sec.

Owing to breeze when the foil was in the circuit the galvanometer needle kept oscillating.

Maximum and minimum readings of galvanometer needle during two minutes:

Max.	Min.	Max.	Min.
88	49	96	61
68	65	106	44
70	66	72	50
80	74	84	70

i. e. the temperature varied from 37·7° C. to 23·2° C.

When the fur was in circuit the reading was almost steady during two minutes 103–6, i. e. 37°–37·7°.

Deflections given when the thermo-junction was placed in

Black fur	109 = 38·4° C.
,, ruffled up by wind	96–106 = 35·4–37·7
White fur	96 = 35·4
Black ostrich feather	95–8 = 35·2–35·8
Grey opossum fur	101–9 = 36·5–38·4
Grebe breast feathers, light coloured	111–19 = 38·8–40·7

Fur and feather-down show very considerable inertia in both taking up and in losing heat, and thus keep up when exposed to the wind and sun a far more constant temperature than a thin smooth black surface.

The adjoined tables illustrate by black fur and cheek temperature readings the radiation effect of the sun, and the difference felt on sunny and cloudy days outdoors and indoors on sunny days, &c.

The first figures contrast sunny calm and rainy calm summer days. The chilly feelings of the rainy day are shown in far less radiant absorption and in higher cooling power; there is also the touch of much colder air.

July day, sunny, calm:

Air	Black fur	Blue flannel sleeve	White flannel sleeve	Dry K.T. cooling power
26° C.	57° C.	52° C.	34° C.	1·5

Sweating profusely in sun.

August day, rain, calm:

14	15	19–20	20	7·3

Chilly sitting in flannel trousers, cotton shirt, and flannel coat.

The next figures contrast two days of approximately the same temperature and cooling power, one sunny, the other overcast by light clouds. The notable effect of the radiant energy of the sun on the surface temperatures of skin and clothes makes a very high cooling power borne with feeling of well being.

September day, south-west breeze, sunny, bracing, glorious day. Sitting on cliff, Irish tweed suit, felt cold on windward side, warm feet:

	Air	Black fur	Sleeve	Cheek	Dry K.T. cooling power
Shade and wind	13·8° C.	22° C.	19° C.	22° C.	28
Sun and shelter	14·5	43	36	30	—

September day, lightly overcast, south-west breeze, very cool and cold feet, sitting on cliff in Irish tweed suit:

13·2	16	20	23	25

On an August day of approximately the same temperature but lower cooling power a rug was required for sitting out on the cliff in comfort.

N.W. breeze, clouded :

Air	Black fur	Sleeve	Cheek	Dry K.T. cooling power
14·5° C.	15° C.	23° C.	27·5° C.	18·6

There was little radiant energy, as shown by the black fur and air temperatures being almost the same.

The following contrast outdoor, indoor, and greenhouse conditions on a sunny August day.

August day. Noon, sunny, strong S. breeze on east coast cliff, ideal conditions, sitting in white flannels and blue flannel jacket :

Air	Black fur	Blue flannel coat sleeve	White trouser	Cheek	Dry K.T. cooling power
22° C.	30° C.	28–29° C.	27° C.	32·2° C.	16

Note the high cooling power, three times that of an ordinary room.

Indoors, shady and sheltered side of house, wide open window, pleasant but dull compared to out of doors :

21	22	26	26	—	4·6

In greenhouse, door and skylights open, sun shining on head and fur through open skylight :

26	57	—	—	38	—

Sun shining through glass which cuts off some of the radiant energy :

—	44	—	—	36 (sweating)	2·5

The sun feels unpleasantly hot and burning to the head in the greenhouse owing to the absence of wind and low cooling power.

The water-jacketed thermopile showed there was less diffusive reflection of the sunlight from the face in the greenhouse than there is in the open, owing to the cutting off of some of the sun rays by glass. It was much pleasanter out of doors owing to the breeze and high cooling power.

September day. In greenhouse :

Air, 23·7° C. Water-jacket, 15° C. Dry K. T. Shade, 5·8. Sun, 3·3.

Face		Deflection of galvanometer	Surface temperature
Face	Sun	11 degrees of the scale	37·5° C.
,,	Cloud	7 ,,	—

Out of doors, shelter of bushes from breeze :

Air, 19·5° C. Water-jacket, 15° C. Dry K. T., 9·3. Sun, 4·7.

Face		Deflection of galvanometer	Surface temperature
Face	Sun	13 degrees of the scale	35° C.
,,	Cloud	9 ,,	—

A series of temperatures were taken by H. E. Roaf in Cairo with a black-bulb thermometer *in vacuo* shaded from the sun by a piece of tin placed 10 cm. above it, and screened by a piece of black cloth placed 10 cm. below to stop upward radiation, and curtains of black muslin to diminish side radiation, through which air could freely pass when the breeze blew. The temperatures thus recorded in September

1918 mid-day varied from 45–51° C.; they show what a high amount of radiant energy is received by a man sitting in such an enclosure, the walls of which become sun-heated in spite of the reflection from the tin.

The radiation thermometer fully exposed to the sun showed temperatures varying from 67 to 73·5° C.

A white muslin screen lowered these figures down to 49·5–55·5, a black muslin screen to 48·5–54. Screens of white over black muslin, white or black sail cloth, and khaki drill lowered the figures to the same extent as the tin screen.

The next figures show the effect outdoors in England of the sun coming out and in, and the uniformity of indoor surface temperatures.

July day. Calm, after thunderstorm, moist. 6.30 a.m.

Indoors:

Air	Black-bulb thermometer	Black fur
°C.	°C.	°C.
19·3	19·2	20·5

Out of doors, very hazy sun:

18·4	23	26·5

6.45 a.m. dull, grey, overcast:

18·8[1]	22·2	22·8

7.15 a.m., sun coming out stronger, very pleasant warmth:

—	31	40

7.30 a.m., grey, overcast:

19	26[2]	22·5

8.40 a.m., sun out, warm:

24	46	51[3]

July day, overcast, cool, gentle breeze.

6 a.m. Indoors the walls, hangings, furniture, and air were all at 16·5° C.

Outside in front of the house:

	Air	Black-bulb thermometer	Black Fur	White fur
	°C.	°C.	°C.	°C.
6 a.m.	11·5	19	18	14
6.30 a.m., sun out	15·5	41	45	—
8.15 a.m., sunny	—	48	48	—

Another July day. 10 a.m.:

Out of doors	Air	Black-bulb thermometer	Black fur	Black pile rug	Black umbrella	White fur
Sunny, very	°C.	°C.	°C.	°C.	°C.	°C.
gentle breeze	—	48	48	38	42	—
,,	18	48	57	50	—	46
,,	27	55·5	58	57	—	—
In shadow	22	23·5	22	23	—	22

[1] The ground, wet leaves, and flowers were at 18° C.

[2] The radiation thermometer is much slower than the black fur (owing to the vacuum) in coming into equilibrium.

[3] Hair of head.

July day, light white fleecy clouds cover the sky; cool breeze:

	Air	Black-bulb thermometer	Black fur	White fur
	°C.	°C.	°C.	°C.
10 a.m.	17	24·5	25	22·5
1 p.m.	19	27	27	23
5 p.m.	19	23	21	19·5
7.30 p.m.	15·25	15·5	14·5	14·5
8.45 p.m.	13	12·5	12·25	12·25

Indoors at 5 p.m. the readings were:

19·5	20	20	—

The radiation from such white clouds has a notable effect on the black fur surface temperature during the height of the day, which vanishes as evening comes on and the sun gets low in the sky.

The black fur is a little higher than the air temperature on October days even when overcast and rainy.

On a very bracing sunny day, freezing in the shade, the readings were:

Air, shade	−1° C.
,, sun	−0·5
Black fur, sun	9–10 varying with breeze.
Black fur held so as to receive sun-glare from the snow	1·5

The following records taken by Capt. H. E. Roaf show the effect of the Egyptian sun on thermometers clothed in black and white muslin:

		Shade	Sun			
Date	Time	Uncovered	Uncovered	White muslin	Black muslin	Remarks
		°F.	°F.	°F.	°F.	
13. 3. 15	14·00	68·8	87·8	91·8	108	Sunny day.
14. 3. 15	14·00	68·5	81·0	89·0	102	Sunny with clouds.
16. 3. 15	14·00	74·5	85·8	86·0	93	Close.
20. 3. 15	13·40	76·5	88·0	92·0	104·5	Bright day.
29. 3. 15	13·45	76·0	97·0	99·0	108·5	Oppressive sunny day.
30. 3. 15	13·45	81·0	93·5	103·0	119·5	Bright sunny day.
6. 4. 15		76·0	83·0	85·0	91·5	Sunny day.
12. 4. 15	13·45	81·0	93·5	97·5	105·0	
13. 4. 15	13·30	94·7	102·5	104·5	106·5	Hot wind.
17. 4. 15	13·30	77·5	87·5	87·0	100·0	Slight breeze.
18. 4. 15	13·45	78·5	86·5	88·0	98·5	Faint haze.
26. 5. 15	12·30	99·0	110·0	117·0	128·0	Hot breezy day.
27. 5. 15	12·30	104.5	122·0	123·5	136·0	Hot day, slight breeze.
6. 6. 15	12·45	91·3	111·5	110·5	117·0	Warm day, cool breeze.
13. 6. 15	12·30	95·3	112·0	116·0	121·0	,, ,, slight breeze.
15. 6. 15	14·30	108·0	125·0	127·0	137·0	Hot still day.
16. 6. 15	15·15	114·0	123·0	124·0	131·0	Slight haze.
21. 6. 15	13·30	90·5	105·5	111·0	120·5	Cool breeze.
22. 6. 15	13·30	89·8	106·5	114·0	123·0	Gentle breeze.
23. 6. 15	13·30	98·8	117·0	120·0	129·0	Hot still day.
24. 6. 15	13·30	91·8	105·0	110·0	117·0	Stimulating breeze.
8. 7. 15	14·30	93·0	112·0	113·0	126·0	Hot day.
11. 7. 15	12·45	93·0	110·0	112·0	125·0	Refreshing breeze.
15. 7. 15		95·5	114·0	114·0	127·0	Cool breeze.
18. 7. 15		98·0	116·0	119·0	132·0	Hot day.
21. 7. 15		94·0	114·0	115·0	125·0	Hot day, no breeze.
31. 7. 15	12·00	92·0	108·0	112·0	122·0	
1. 8. 15	12·15	93·0	105·5	110·0	122·0	
2. 8. 15	12·15	92·5	108·0	109·0	125·0	Hot sunny still day.
3. 8. 15	12·00	90·5	105·0	108·0	130·0	
5. 8. 15	11·30	89	105·0	112·0	130·0	

The maximal difference due to the absorption of the radiant energy of the sun by black is 22° F. The white muslin and uncovered readings approximate except on breezy sunny days, when there may be 5–10° F. difference, the white muslin impeding the cooling action of the breeze.

Using thermometers whitened or blackened, observations were also taken in Egypt by Capt. H. E. Roaf:

Effect of colour on absorption of sun's rays

Date	Temperature in laboratory	Dry bulb in sun		Dry bulb in test tubes in sun		Wet bulb in sun		Wet bulb in laboratory	
		White	Black	White	Black	White	Black	White	Black
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
12. 8. 17	92	101	110	112	142				
13. 8. 17	92	101	108	111	142				
14. 8. 17	93	104	113	116	141				
15. 8. 17	92	101	108	114	137	84	85	81	81
16. 8. 17	91	99	106	111	138	82	84	80	80
17. 8. 17	92	104	109	118	141	82	84	81	81
18. 8. 17	95	106	114	119	143	81	82	79·5	79·5
25. 8. 17	92	102	113	116	138	81	82	78	78
12. 10. 17	85	—	95	—	130	—	—	—	—
18. 10. 17	84	93	105	107	134	75	—	71	—
19. 10. 17	82	90	94	—	127	—	—	—	—
9. 12. 17	63·5	—	76	—	100	—	—	—	—
11. 12. 17	66	—	78	—	101	—	—	—	—
12. 12. 17	69·5	—	85	—	115				
15. 12. 17	65		80		110				

9. 12. 17 Sun warm, but not unpleasantly hot.
11. 12. 17 Sun hazy, but felt warm on face.
12. 12. 17 Sun feels decidedly hot on head.
15. 12. 17 Sun warm, but not uncomfortably hot.

Exposed to the sun and breeze the blackened bulb registered 4° to 12° F. higher. Exposed to the sun in still air the blackened bulb registered 22° to 31° F. higher. The sun acting on the blackened bulb in still air heated it 36° to 41° F. above the whitened bulb in open air, while the white bulb in still air was heated 10° to 14° F. above the white bulb in open air. Similarly the effect of sun on the body dressed in black and in still air is most potent. The whitening or blackening makes little difference to the wet bulb temperature; the black is 1° to 2° F. higher in the sun.

The following observations were made in front of a white house, Loughton.

July day, sunny, very little breeze. Temperatures exposed to sun.

	Air	Black-Bulb thermometer	Black fur	White fur
	°C.	°C.	°C.	°C.
6 a.m.	16	26	26	—
7 a.m.	19	39	40	—
8 a.m.	20	43	43	—
9 a.m.	23	46	46	35
11 a.m.	24	50	50	39½
1 p.m.	26	51	50	38½
Shade.				
3.30 p.m.	26	31	30	29

On a calm sunny day the surface temperature of black fur and the black bulb in vacuo keep closely together. The surface of black clothes absorbing like black fur becomes 20° to 25° F. hotter than the air. White fur, on the other hand, has considerably lower surface temperature, but white fur is warmer in the depths, while black fur is cooler in the depths than on the surface. For example:

July day, sunny, calm.

	Surface °C.	Deep °C.	
Black fur	57	52	
Grey opossum fur	41	48	
White fur	41	51	
,,	34	46	(another occasion)
Cotton wool	41	45	
Shining white smooth breast feathers of grebe	40	51	
Dark fur of live rabbit	57	47	
,, ,, ,,	55	49	
Black hair of boy's scalp	48	—	
Grey hair of man's scalp	38	—	

The light that penetrates between the fibres of fur, cotton wool, or feathers is reflected to and fro and absorbed. The surface reflects more in the case of white, and therefore the temperature is lower than black.

The effect of radiation on various garments is shown by the next figures.

July day, sunny, gentle cool breeze out of doors, 6.30 a.m.

Air	Black-bulb thermometer	Black fur	White fur	Glazed white collar	Soft white collar	White hand-kerchief	White flannel	White flannelette
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
16	34.5	35	27	17	19	19.5	22	21.5
	9.30 a.m., same conditions.							
21	51	55	35	26	30	—	34	34
	10.30 a.m., overcast, light clouds, small blue patches.							
19	27	27	23	22	22	22	23	23
	1.30 p.m., sunny.							
—	—	57	35	—	—	30	—	—

July day, sun too hot to sit in comfortably in spite of cool breeze.

Black tie	White shirt	Dark grey coat	Cheek sweating
52	41	49	34

The difference of surface temperature in overcast and sunny conditions of various garments is shown by the following:

July day, 12.30 p.m., overcast, light grey and white clouds.

Black fur	White fur	Khaki soft Jaeger rug	White cotton wool	Fine white linen	Black ostrich feather
27	25	26	25	23	27
1.30 p.m., sunny, cooling breeze.					
57	35	41	36	30	41

The following observations show that dark-coloured flowers have higher surface temperatures in the sun.

June day, sunny, little breeze. Noon.

Black-bulb thermometer in sun	51° C.
Dry bulb exposed to wind	26.5
Surface temperatures of flowers and leaves.	
Scarlet geranium	34
Yellow calceolaria	33
White pink	30
Pale pink peony	31.5
Green peony leaf	29
White violas	29
Blue ,,	30
White rose	30
Red ,,	32
Orange marigold	33
White single rose	29
Dark claret rose	34
Orange escholtzia	31
Pale pink Canterbury bell	30
Dark blue ,, ,,	32
Sweet William, purple centre	33
Green leaf of peach on wall	32

Surface and body temperature of live rabbits exposed to sun. July day, sunny, calm.

Rabbit I.	Black nose	Dark hair of back		Ear folded	Rectum
		Surface	Deep		
	51° C.	57° C.	47° C.	40° C.	39° C.
Rabbit II.	Sun out	Overcast			
	55	26			
A piece of black fur	56	26			
Rabbit III.	White fur	Yellow brown		Ear folded	
	37° C.	55° C.		38° C.	

Radiation is proportional to the 4th power of the absolute temperature, and the loss due to convection is greater from the hotter surface. The rate of absorption of the sun-rays by black fur, therefore, is much greater in proportion to that by a smooth white surface than is shown by the above figures.

CHAPTER VIII

Acclimatization to Insolation.

Aron killed monkeys by exposing them to the sun in the Philippines. They died with body temperatures reaching respectively 43·5, 42·7, 46·3, and 44·8° C.

Shade by an umbrella or a board prevented death. So did a brisk current from a fan. Death was due then to heat-stroke and not to any lethal effect of light rays, a conclusion confirmed by the fact that monkeys enclosed in boxes with the head only exposed suffered no inconvenience, although their scalp temperature reached 47° and the brain was exposed to any possible penetration of rays.

Schilling[1] found the subcutaneous temperature of a white rabbit in the shade to be 38·4°. In the sun in 30 min., 40·4°. The skin was shaved—in 30 min., 41·5°. The shaved skin was covered with black cotton—in 25 min., 42·8° ; this was removed and the thermometer fell to 39·6°. The skin was blackened with carbon—in 10 min., 42·4°. On the day of these observations the air temperature was 26°, the black-bulb thermometer 46·7°.

P. Schmidt[2] exposed a white and a black rabbit to the sun. The rectal temperature rose from 38·5° to 39·5° in the white and to 40·2° in the black. If the neck alone was exposed the rise was only 0·3°.

According to Aron, one monkey's subcutaneous temperature reached 54° C., another's 48·3° when sun-exposed. Such temperatures must produce burns of the subcutaneous tissues ; possibly products of cellular disruption add their influence (as in wound shock) in producing heat-stroke.

Black, grey, and white rabbits were exposed. The subcutaneous temperature of the black reached 44·2° after 36 minutes' exposure to the sun, the grey to 52·8°, the white to 41·0°. The black died after the exposure.

In another experiment the black reached 47·8° after 31 minutes' exposure to the sun and died, the grey 44·9° after 86 minutes' exposure and died, the white 45·7° after 90 minutes' exposure and recovered when then put in the shade.

Castellani and Chalmers, in Colombo, exposed rabbits with their heads shaved to the sun in a yard, and others in a box protected by coloured glass. Those in the yard died, the others survived, although feeling very hot to the hand. They concluded that there are active chemical rays which cause death. Against this conclusion must be put the fact that coloured glass absorbs heat and light rays. The rabbits were thus protected from heat-stroke.

H. E. Roaf observed the effect of coloured gelatine screens on the radiation thermometer exposed to the sun in Cairo in the month of September. The readings were as follows:

Fully exposed	Gelatine Screen		
	Yellow	Red	Blue
°F.	°F.	°F.	°F.
70·5	63·5	60·0	—
73·5	66·5	62·5	—
72·5	—	60·5	62·0
73·0	—	62·0	63·5

[1] *Arch. f. Schiffs- u. Tropen-Hyg.*, 1909, **13**, 1.
[2] *Arch. f. Hyg.*, 1903, **47**, 262 ; 1908, **65**, 17 ; 1909, **65**, 1.

The screening with coloured glasses must have similarly reduced the absorption of the sun-rays, and the heating of Chalmers and Castellani's rabbits. The fur was probably heated 10–20° C. higher in the case of the rabbits fully exposed to the sun in the yard.

The evidence seems conclusive that sunstroke is due to overheating of the body.

Shaklie [1] carried out a careful and valuable series of observations on monkeys, and disproved the two assertions of Aron, first, that these animals have no sweat glands; secondly, that they must die if exposed to the sun for over an hour in Manila. He says the conditions which favour the production of heat-stroke in the monkey, in addition to a hot sun, are proximity of a large hot surface—ground or roof—high humidity, low wind velocity.

Monkeys on a suitable diet, e.g. boiled white rice and ripe bananas, may be acclimatized to exposure to the sun if this is made gradual, the acclimatization depending on an increased sensitivity in, and efficiency of, the sweating mechanism. A monkey so acclimatized may be safely exposed for even seven hours to the Manila sun. A small dose of atropine, by stopping sweating, causes death. Rabbits which do not sweat cannot be acclimatized. The white man standing high off the the ground, having less body hair and greater power of sweating, can acclimatize himself readily to perform physical labour exposed to the tropical sun and without immoderate sweating, provided he is not over clothed, eats a suitable light diet, and gradually accustoms himself to work in the sun.

Shaklie found that owing to the cooling effect of wind and drier air not affecting the black-bulbed thermometer this might be considerably higher on days when monkeys survived than on days when monkeys died from heat-stroke. Exposure to the sun on a hot surface may make a great difference, as shown by the ground-surface temperatures in the following table. The wind near the ground is much less. The effect of the wind dropping, after noon, on the rectal temperature is shown in the table.

Time	Air temp. on ground	8·5 cm. above ground	1 m. above ground (in house)	Body temp. of monkey	Time	Wind	Relative Humidity	Sunshine
	°C.	°C.	°C.	°C.				
7.55 a.m.	—	—	22·5	37·4	9 a.m.	0·0	62	7.31–11.0
9.43	38	32	28·5	39·8	10	2·5	60	—
10.55	42	32·5	29	39·4	11	5·0	50	11.25–11.45
					12	3·0	54	11.46–11.47
1.17 p.m.	46	35	32	40·5	1 p.m.	0·5	58	12–2.45
2.30	44·5	35	32·5	41·7	2	1·5	58	
3.15	37·5	30·5	29	42·0	3	0·0	50	—
4.0	34	29·5	29	39·0	4	2·0	68	3.58–4.10

The black-bulb vacuum thermometer registered about 50° C. when the sun was out.

This monkey, after acclimatization, never showed a maximal temperature in the sun over 40·1°, averaging in the sun 39·7°, probably a normal maximum for monkeys.

[1] *Philippine J. Sc.*, 1917, **12**, 1.

CHAPTER IX

Body and Skin Temperature in the Tropics and the Effect of Exercise and Sweating.

Under normal open-air conditions of a temperate climate, the cutaneous tissues and the extremities are cooler than the deep parts of the body. In tropical conditions, when heated by work or in hot baths, all parts of the body may reach or exceed normal body temperature. There is in consequence an increase of the total oxidation process, as the living substance has a relatively high temperature coefficient for its reactions.

The normal rectal temperature of people undergoing open-air treatment in cool weather is about 36·2° C. (97·2° F.) at 9 a.m., and 37° C. (98·6° F.) at 6 p.m. The mouth temperature is about half a degree lower.

The temperature may be made higher by night and lower by day by keeping monkeys awake and active by night and in sound- and light-proof cages by day ;[1] so too in the case of man tested in an arctic winter shelter (Lindhard).

The average temperatures[2] of the abdomen, when wool was worn next the skin, are minimum 35·3° at 7 a.m., maximum 36·9° at 9 p.m.

How the surface temperature of the cheek varies from 35° C. in a warm factory to 20° C. in a cold wind has been shown in the records given in Part I.

Benedict[3] found the skin temperature varied from 28·1° in the calf to 34·7° in the waist (clothed). In an artist's model after 2½ hours' exposure, naked, in a room at 14½° C., there was a difference of 10·6° C. between the highest and lowest parts; at 25·8° C. the difference was 5·4° C. ; at 30° C., 4·2° C.

The average temperature taken in the stream of urine of 83 soldiers on a hot August day—temperature 31·5° C. (88·7° F.)—was 37·58° C. (99·56° F.) ; the maximum 38·22° C. (100·8° F.) ; the minimum 36·78° C. (98·2° F.) ; the men wore light khaki, and the observations were taken while they were resting about an hour after dinner, 3–4.30 p.m.

The body temperature is from half to one degree centigrade higher in tropical than in temperate climates.

The Italian standard axillary temperature is 37·3° C., the Norwegian 36·4°. Travellers from Cape Horn (freezing) to Calcutta (40° C.) showed an elevation of body temperature of 1° C.

How notably muscular exercise raises the temperature, and therefore contributes to danger of heat-stroke in the tropics, is shown by the following :

[1] Simpson and Galbraith, *Tr. Roy. Soc. Edin.*, 1905, **45**, 65.
[2] Pembrey, *Guy's Hosp. Rep.*, 1902, **57**, 308.
[3] *Proc. Nat. Acad. Sc.*, 1919, **5**, 218.

Pembrey observed in himself after a bicycle ride of about two miles in 15 min. against a strong cold wind—

Temperature of air, 5° C.

36·78°	Mouth
37·56	Urine
37·67	Rectum

Ditto in 13 min. against no wind. Air, 1° C.

36·95	Mouth
37·72	Urine

Ditto in 11 min., no wind. Air, 5° C.

37·22	Mouth
37·83	Urine

Ditto slow pace. Air, 27·75° C.

38	Mouth
38	Urine

A runner had a body temperature of 39·5° C., that of dogs struggling rose from 38–41°, a dog thrown into tetanic convulsions 44° (Richet).

In a hospital cup-tie football team on a cold winter day the rectal temperature was 102–103° F. at the end of the game. That of an athlete (not in good training) after a three-mile race, 105° F., and it was some hours before this fell to normal.[1]

Walking in tropical humid heat raised the rectal temperature to 101–103° F.[2]

On exposure to the sun the temperature of the human skin rises until sweating breaks out; it then remains below body temperature. For example, the skin surface temperature of the forehead may rise to 41° C. and then sink to 35° C.

Zuntz records a man who did not sweat. To work in the sun he had frequently to wet his shirt. So with the caribou; to get work out of this animal, in the sun, it has every two hours or so to be bathed in water (Woodruff).

The shielding power of evaporation is shown by the effect of tracheotomizing dogs. The rectal temperature of dogs tracheotomized and put in the sun rises from 39° to febrile heights, e.g. 42–44°. Unable as they are in such cases to evaporate water from their mouth and tongue, their respiration markedly increases, their pulse greatly quickens, saliva drops from their mouths, the mucous membranes becoming cyanotic. If saved by cooling there may be disturbed orientation owing to damage of the brain by over-heating; a dog may restlessly run round and knock its head into the wall.

Three rabbits in the sun lost 14, 12, and 23 grm. of water per hour and kilo against 2, 3, 2 in the shade.

In man the water loss may reach the figure of 1 kg. per hour (nearly two pints). The body weight is a measure of this loss if no food or drink is taken or excreta evacuated.

[1] L. Hill and M. Flack, *Proc. Physiol. Soc.*, Mar. 27, *J. Physiol.*, 1909, 38.

[2] Young, Breinl, Harris, and W. A. Osborne, *Proc. Roy. Soc*, 1920, **B. 91**, 111. Walking 3½ miles per hour gives a heat production of about 300 calories per hour in place of 70—the resting value.

The volume of urine excreted is smaller, the specific gravity higher, the total nitrogen and sodium chloride less in the tropics, as shown by the following table giving the average of 25 persons, some of whom were manual and some sedentary workers, in Townsville.[1]

	In Townsville.	European Standard.
Quantity	782 c.c.	1,500 c.c.
Specific Gravity	1·025	1·015 to 1·020
Total Nitrogen	10·4 grm.	16 grm.
Sodium Chloride	7·00 grm.	15 grm.
Phosphates	1·73 grm.	2 to 3·5 grm.
Freezing point	−0·935° to −2·259° C.	−0·87° to −2·71° C.

An American's arm, cheek, and chest skin temperature rose in the sun after 30 min. exposure to 36·9°, 36·2°, and 36·5°; a Filippino's to 36·1°, 35·4°, and 35·4°.

Out of 14 observations, 12 showed a lower skin temperature in the case of the Malay. The highest American cheek temperature was 37·4°, chest 41·8°.

'The naked human skin,' says Aron, 'if exposed to the sun, rises quickly to 36°, maximum 37°. Sweat breaks out then and the temperature falls.'

In the dense black hair of a Filippino the thermometer reaches on insolation 45° in 30 min. and may reach 50° in an hour.

Schilling placed a thermometer between the teeth and cheek of a man. It registered 36·6°; and with the face exposed to sun 37·05°, showing that little local heating takes place while the circulation is active. The black-bulb thermometer registered 55°.

The forehead secretes earlier than the arm, and its temperature falls earlier and generally lower.

Rubner could not find greater evaporation from the negro at high air temperatures, although he keeps cooler than a European.[2]

'It is a matter of general observation', says Aron, 'that, at a time when the white man is perspiring over his entire body, and the sweat is dropping from his face and forehead, the brown man shows only a fine, velvet-like layer on his skin. It is not the sweat which we see, but that which we do not see, which exerts the cooling effect; in other words, the water evaporated, not the water secreted, is of value.'

'Hypersecretion is useless, it deprives the body of water. The brown man is superior to the white in this economy of sweating.'

Freer states that the Malay has 12–15 per cent. more sweat glands than the white; the negro also appears to have more.

'I have noted,' writes Mr. A. Ellis to me, 'when in the tropics, that fair white men seem to sweat more profusely, and in larger drops, than white people with a dark complexion, and a great deal more than the coloured negroes and Indian coolies. The Indian coolie does not appear to sweat as much as the negro, but this can be explained by the fact that the former works naked (except for a loin

[1] Breinl and Young, *Med. J. Australia*, 1919, May 3, 10, and 17. The average percentage of sugar in the blood of Europeans in the Indies is 30–75 per cent. higher than the figure generally accepted as normal in Europe. Langen and Schut, *Med. v. d. Geneesk. Dienst in Nederl. Indie*, 1918, 3, 1.

[2] R. Stigler, *Arch. f. d. ges. Physiol.*, 1915, 160. 445.

wrapper) and oils his skin with coco-nut oil, while the latter at most removes his shirt and works in trousers and never oils his skin.' Oil is effectual in reflecting sunlight from the skin.

Augustin Kramer [1] says:

The pigment of the Samoans being a protection against the destructive chemical action of sunlight, their skin vessels can safely flush beneath it. The white sweats at rest, while the Samoan glows like a febrile white and, freely losing heat by convection and radiation, needs to sweat only on exertion. The Samoans oil their skins before diving and fishing in the sea, and their skin glows in their houses after washing the oil off in fresh water. They feel acutely the cold in the higher regions of the island. Their heat-regulating mechanism is not trained as in the white to resist cold. The white has a rougher, thicker skin, allowing less conduction of heat, a more powerful vaso-constrictor mechanism, and cutaneous tissues trained to withstand colder and more bloodless conditions. His heat-producing mechanism too is tuned up to meet cold.

[1] *Die Samoa Inseln*, **2**, 41.

CHAPTER X

Heat-stroke.

Heat-stroke results from the rise of body temperature to a critical height, such rise taking place owing to inability to control the body temperature by, or through exhaustion of, the heat-regulating mechanisms, under the conditions of the environment. In stagnant warm moist air the evaporation of sweat does not suffice to cool the body, which becomes surrounded with a layer of stationary air saturated and almost at body temperature. It is in indoor places that such conditions arise, particularly when air is entangled in the clothes and between the bodies of crowds, e. g. the Black Hole of Calcutta. In such conditions heat-stroke occurs with the skin moist.[1] Heat-stroke is more likely to occur in cities, where buildings confine the air and allow it to become heated by absorbing sunlight, and moisture laden; so too in close valleys. As it takes some time for the body to warm up to critical febrile heights, such conditions are endured in crowded meetings in hot close weather with nothing worse than feelings of exhaustion and the fainting of a few whose circulatory system is unequal to meet the strain.

In the case of the Black Hole of Calcutta, on one of the hottest of Indian nights, 146 English prisoners were confined in a small cell, large enough comfortably to take two. There were two barred windows and the cracks round the bolted door through which air could enter. All but a score of the prisoners died during the night of heat-stroke. A survivor told how within a few minutes of entering the dungeon every man was bathed in sweat, clothing was stripped off, breathing became laboured, thirst grew intolerable, vain efforts were made to force the windows and door, ending in riot and delirium. Those who fell to the floor were trampled and crushed to death, and delirium ended in stupor.

Similarly, men have died from heat-stroke when confined in the cabins of ships. Here is the description of the conditions found on a slave ship. 'The space between-decks was divided into two compartments 3 feet 3 inches high, the size of one was 16 by 18 feet, and of the other 40 by 21 feet; into the first were crammed the women and girls, into the second the men and boys; 226 were thus thrust into one space 288 feet square, and 336 into the other space 840 feet square, giving to the whole an average of 23 inches, and to each of the women not more than 13 inches.' The temperature in the shade on deck was 89° F. There were grated hatchways, but the greater part of the cells were roofed by the deck. 'The heat of these horrid places was so great, and the odour so offensive, that it was quite impossible to enter

[1] See Gauss and Meyer, *Am. J. M. Sc.*, 1917, **154**, 554.

them had there been room.' On liberation the slaves swarmed upon deck 'like bees from the aperture of a hive', 517 of them totally nude. (Some children were found lying in a torpid state in the cells.) 'When water was brought they all rushed like maniacs towards it.'

The space in the cells was so low that the slaves sat between each other's legs, and stowed so close that there was no possibility of their lying down, or at all changing their position by night or day. The ship had been out 17 days, during which 55 had been thrown overboard. Starvation, which lessened heat production, and the wind of the open seas blowing over the grated hatches must have been the two factors which allowed any to be conveyed over to Brazil alive.

The slaves just landed from a Guinea ship appeared 'as a set of scarcely animated automatons', 'walking skeletons covered over with a piece of tanned leather'. Yet the recuperative power of the negro is so great that after ten days' or a fortnight's good feeding many of these physical wrecks were in prime condition for the slave market.[1]

In tropical countries thick-walled stone buildings and underground places are used as shelters from the heat of the day; the sun has not time to warm them up by day and they cool by night. The tropical house, built with a double roof (ventilated) and screened by verandahs, is shut up by day and fans and punkahs are used. Fanning and wetting the skin will not only relieve discomfort but counteract the onset of heat-stroke in those whose heat-regulating mechanism becomes exhausted, so long as the wet bulb is below body temperature. Heat-stroke inevitably results if exposure be continued to hot moist atmospheres with a wet bulb approximating to, or above, body temperature. Such conditions in the open air are fortunately very rare, and a compressed-air douche (the air escapes cool and dry), and drinks and baths of cool or iced water, are the means of counteracting them.

It must be borne in mind that the vapour tension rises very rapidly with increasing temperature, and so by becoming febrile the body can evaporate sweat when the wet bulb reaches body temperature; wind so enormously helps the rate of evaporation that with the help of it a man may safely pass through a temporary exposure to such conditions, febrile, it is true, but without reaching a body temperature critical to existence. The warming up of the mass of a man's body, 70–80 kg., requires time.

Thus miners in excessively hot moist mines get through a shift, the work naturally being most inefficiently done.

The limits of a man's power of accommodation in still air are passed when the wet bulb exceeds 88°–90° F. (31·1°–32·2° C.), even when the man is stripped to the waist and doing no work.[2] In air moving at 2 miles an hour the critical wet bulb temperature became 93° F. (33·9° C.). Under such conditions the body temperature rises steadily, the pulse becomes rapid, and there is profuse sweating, accompanied by dyspnoea and exhaustion. When muscular work is performed the rise is much more rapid and begins even at a wet-bulb temperature of 80° F. (26·7° C.). Wet-bulb readings of 90° F. are not uncommon at Beaufort, North Borneo.[3]

[1] Cit. by Sir Harry Johnston, *The Negro in the New World*, London, 1910, p. 85.
[2] Haldane, *J. Hyg.*, 1905, **5**, 494.
[3] J. W. Gregory., *J. Scot. Meteorol. Soc.*, 1912, **16**, 7.

The following observations exemplify the effect of hot moist atmospheres:

Pembrey and Collis[1] observed in a paper-mill with

Dry bulb		Wet bulb
101° F.	and	88° F.

after 1 hour exposure their rectal temperatures to be:

100·5° F.	100·8° F.
Pulse	
118	116

In a small chamber heated by a coke brazier and steam pipe, with dry bulb 100° F., wet bulb 85° F., I found the rectal temperature of a workman wearing jersey and trousers was 100·6° F.; stripped to the waist it became after moving 100 bricks 101·1° F.

Water was then evaporated in the chamber and the dry bulb became 108° F., and wet bulb 103° F. The rectal temperature of the workman rose to 102·6° F.

After 45 minutes in the chamber with dry bulb 118° F., wet bulb 90° F., the rectal temperature of a workman wearing an oxygen-breathing apparatus was 100·3° F., while the skin temperature taken under a canvas strap was 101° F.

In the case of another man dressed in ordinary clothes the rectal temperature was 101·1°, and became 102° after moving 100 bricks with dry bulb 114°, wet bulb 84°. Haldane found in one case the rectal temperature rose 3·5° F. in an hour when resting in a chamber with wet bulb at 87° and no fan.

After 78 minutes' exposure in a hot moist room

Dry bulb	Wet bulb
94·8°–100·3° F.	98·2°–96° F.

W. A. Osborne[2] and others recorded the following:

Rectal temperature rose from	Pulse-rate rose from		Systolic blood pressure rose from
	Lying down	Standing	
99·5° to 103·0° F.	72 to 112	72 to 168	112 to 142.

In the case of one individual, fainting resulted after 40 minutes' exposure to dry bulb 100·1°–100·7° F., wet bulb 98·7°–102° F. The rectal temperature had risen to 102° F. and the pulse-rate to 132.

Walking in a tropical climate—e. g. wet bulb 75°–80° F.; dry bulb 80°–90° F.—may raise the rectal temperature 2°–3° F. and send the pulse-rate up to 140–160.

Mr. Eric Davies reports to me that in a mine in Brazil, after sitting down for 20 minutes in a stagnant atmosphere with dry bulb 101° F., and wet bulb 93° F., his mouth temperature was 100·4° F. and his pulse frequency 120. The miners have worked in this mine in wet-bulb temperature varying between 80° and 93°, doing 8- and even 12-hour shifts. The mine is now being cooled by the installation of a special plant.

While in saturated air above body temperature heat-stroke on continued exposure is inevitable, in hot dry air, on the other hand, it affects only those whose sweating mechanism is insufficient or ex-

[1] *Journ. Physiol.*, 1911, **43**, Proc. Physiol. Soc., Oct. 21.
[2] *Proc. Roy. Soc.*, 1920, **91**, B. 111.

hausted from fatigue, or lack of drink; intemperance and infection contribute greatly to fatigue of the heat-regulating mechanism.

Blagden and Fordyce only remained 15 minutes in a damp room heated to 129·9° F. (54·4° C.), and the body temperature rose in this time to 100° F. (37·8° C.); on similar exposure in a dry room heated to 239·9°–260° F. (115·5–126·7° C.), and in which beef steaks were being cooked, the heat of the air did not raise the temperature of the body above normal. If, however, occupation of such an oven were to continue, the sweating mechanism would eventually fail and heat-stroke result.

Hale White found a few minutes' immersion in a bath at 108° F. increased the evaporation of water from his skin 80 times. This shows the strain put on the secretory mechanism.

Since at each air temperature above blood heat the bodily gain of heat from the air by convection increases as the strength of the wind

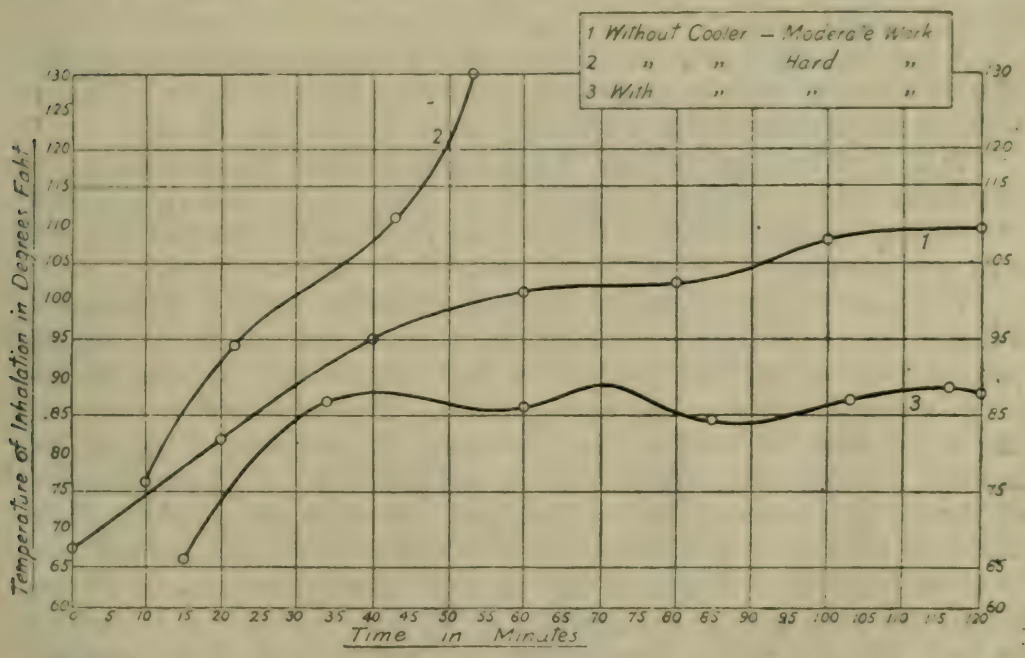


Fig. 15. Proto mine-rescue breathing apparatus worn with and without cooler. The rise of temperature of the inhaled air shown in Curve 2 caused the wearer to become exhausted, the air being heated by the caustic soda in the breathing bag. The cooler consists of a vessel containing crystals of calcium chloride which absorb heat on melting.

increases, and since the human body cannot perspire above a certain maximum rate, there must, at least on theoretical grounds, correspond a certain critical value of the wind velocity, which if exceeded must produce a net gain of heat to the body. The less the air exceeds body temperature, the higher will be the critical wind velocity.[1]

With an infinite wind, according to the wet 'kata' formula, whatever the dryness of the air the kata-thermometer would gain heat if the air temperature were over 36·5° C.

At an air temperature of 45° C., and relative humidity 40 per cent., the wet kata formula indicates a warming up in a wind of 9 metres per sec., and a cooling in a calm.

Experimental observations require to be made both to test the

[1] C. Normand, *Proc. Roy. Meteorolog. Soc.*, Nov. 19, 1919.

formula of the wet kata under such conditions, and on heat-loss of man and animals, so as to settle the viable limits of wind and temperature. The simoon in the desert kills by the warming effect of wind, the skin being dried by evaporation exceeding perspiration. Arabs overtaken by a simoon crouch down on the ground and cover themselves with clothes to prevent the drying and warming-up of their body by the hot wind and dust. The breathing of hot air helps to cause heat-stroke. Thirst enhances the danger. Stokers who are on duty 4 hours in the draught of a furnace, and then rest and get plenty of drink, rarely suffer from heat-stroke.

Little if any concentration of the blood takes place normally from sweating, either in hot rooms or walking in the tropics. Hunt[1] found no change in the percentage of haemoglobin in the blood, and concluded that all the water excreted in the sweat comes from the tissues or gut. W. A. Osborne and others[2] find evidence of a very slight concentration.

A hot desert wind, by drying the skin, also enhances the absorption of sun energy, which is normally checked by the layer of sweat. Clothes are then useful as a screen; so too in furnace rooms.

While sunburn gives rise to local and general symptoms just as any other burn, sunstroke is a form of heat-stroke, and results from the absorption of radiant energy plus an inadequate heat-loss owing to a close atmosphere. There is no sunstroke in Alpine resorts.

The normal production of heat of a resting man is balanced by a loss from the surface of his body of about 1 millicalorie per sq. cm. per sec.; the extra heat production due to work may require the loss of 3 millicalories per sq. cm. per sec. The absorption from the unclouded sun by a black surface equals 2 calories per sq. cm. per minute, say 30 millicalories per sq. cm. per sec. Suppose one-third of the body is exposed to the sun, and the skin and clothes absorb half and reflect the rest, then 5 millicalories per sq. cm. per sec. may be absorbed. If dark clothes are worn the absorption may be 10, and if half the body surface is exposed 15. A man working in the sun, or in front of a furnace, is then exposed to great danger of over-heating, and requires screening from the radiant energy or a most active ventilation.

The extreme variation of surface temperature of steel tanks observed in the Persian Gulf in the course of 24 hours has been from 26° at night to 185° F. in the sun.

The conditions for producing sunstroke are given by soldiers heavily accoutred marching in close column in the full blaze of the sun on a calm day. The air surrounding their sweating bodies becomes heated and saturated, while the sun energy is absorbed by the fabric of the clothes.

Pembrey pointed out that unsuitable clothing is one of the most serious disadvantages with which the soldier contends. The athlete suitably clothes himself, his garments allow freedom of movement, are scanty and loose enough to secure loss of heat. The soldier on the march is hampered by a tunic of thick material fastened right up to the neck, by tight belts and straps, and the haversacks and accoutrements

[1] *J. Hyg.*, 1912, **12**, 479. [2] *Proc. Roy. Soc.*, 1919, **B. 91**, 111.

they support. The heavy load he carries disturbs the balance of the body, and not only entails much additional muscular work, but interferes with freedom of breathing and natural ventilation of the clothes.

Open order and the stripping-off of clothes, an unheating diet and adequate supply of water, and frequent rests for cooling-off are the safe measures for marches in the sun on close days.

Horses loose in a field never are affected by sunstroke, but at work, particularly in close city streets, they are frequently overcome. In the days of the horse omnibus the companies knew the value in hot weather of cold water douches and rubbing down at various stages of the journey. The rectal temperature of horses may be raised 3°–4° F. by hard work.

Drivers know the danger of rapidly driving a flock of sheep on a close damp day. On a cold day a cloud of condensed vapour is visible over a flock, or column of soldiers.

Prostrations from heat appear in New York with a temperature of 31·5° C. and 66 per cent. relative humidity. The untrained and those with instability of the vasomotor system easily become exhausted from effort in warm weather. The nervous system as well as the muscles demand more blood, the skin, too, must be supplied, and the sweat glands flushed so as to discharge the excess of heat produced. The dyspnoea in untrained men appears to be chiefly cardiac in origin; the heart is unable in them to meet the demand made upon it, and the nervous system fails for want of oxygen. Training develops the power of regulation of the vascular system, and effects the production of work with the least amount of body heat. The prophylaxis in the case of the troops in Mesopotamia included the wearing of topees, spinal pads, dark glasses, and orders preventing men being put on duty in the heat of the day, e. g. from 10 a.m. to 4 p.m.

A spinal pad is of benefit in so far as it is made of material containing air, and therefore of very low conducting power. It shields the spine from the sun, covering it with a mass of material slow to warm up. This may be necessary when men bend to work, e. g. in digging. A white flapping robe is all that is needed for the upright position, but such a robe becomes drawn tight to the back in bending. Then some means are required to keep an air space between the skin and garment.

If the local sweating mechanism over the spine does not suffice to keep moist the skin exposed to the direct action of the sun, then local heating will take place; if the circulation were not sufficient to convey the heat away the spinal cord might possibly become raised in temperature owing to the local, not general, rise of body temperature. Observation is required to settle this question.

The screening effect of pith helmets is illustrated by observations made by Major C. F. Wanhill, in Mhow, India. He fixed self-recording thermometers inside the helmets, which were placed on posts. He proved that reflected heat from the ground and walls raised the temperature inside the helmets considerably, and that wind had a considerable influence—for example:

	Inside helmets	Dry bulb	Wet bulb
Calm	103·6°–108·2° F.	91° F.	68° F.
Strong wind	95°–98°	88°	69°

With the helmets on soldiers sitting in the sun near a wall the readings were :

Helmets	Dry bulb	Wet bulb
109·4°–111° F.	87° F.	68° F.

The men made no complaint of the helmet being hot.

Walking about in the open, strong north wind :

100°–105° F.	86° F.	69° F.

In closed boxes of straw-board, lined with red or white cloth and exposed to the sun, photographic paper was unaffected. No light rays penetrate the wall of the helmet, and dirty white linings are then just as good as red linings. The point to attend to is the ventilation, not the colour of the lining of the helmets.

The view that heat-stroke is an infective disease (sciriasis) was brought forward by Sambon [1] and has been supported by Manson. Sambon asserted that cases of sunstroke are mistaken for cases of syncope, delirium tremens, cerebral haemorrhage, tuberculous meningitis, or cerebro-spinal fever. 'Heat-stroke as a clinical entity is non-existent', says a writer in 1918,[2] 'and is nothing more than a symptom on a hot day of malignant tertiary malaria'. Sambon's contention is disproved by physiological and clinical evidence, by such evidence as H. C. Wood [3] adduced in 1886, 20 years previous to Sambon's paper.

Rogers [4] worked out the data for the temperature and moisture of the air at the time of occurrence of 363 cases of heat-stroke in India. A very large majority of the cases occurred during the hottest months, and of the onsets during the hottest period of the day. Moist heat was an important factor. In 8 out of 14 cases there was no evidence of malaria.

In spite of such evidence, in his book on Tropical Diseases, Manson ascribes heat-stroke as probably due to the toxin of a microbe, and yet says the way to cure it is to cool the patient with ice—a confused sequence of ideas which must be attributed to the common observation that malarial and other infected patients have a disordered heat-regulating mechanism and are far more sensitive to climatic conditions.

In the Mesopotamian Expeditionary Force some heat-stroke cases were complicated by malaria, and the hyperpyrexia in these was partly due to malarial attack. Such cases have a deranged heat-regulating mechanism, and therefore equally with the intemperate are more susceptible.

M. S. Pembrey [5] analysed the records of 50 cases which were reported from the British Army in India.

In 25 cases the wet-bulb temperature of the air was 80° F. or over ; in 8 cases 84° F. or over ; the lowest wet bulb was 67·8°.

In 23 cases muscular work in the sun was a possible factor, and in 12 of these the evidence of this was good.[6]

[1] *Brit. M. J.*, 1898, i. 744 ; 1899, ii. 650.
[2] Milner, *Brit. M. J.*, June 8, 1918. [3] *Pepper's Syst. of Med.*, 1886, **5**, 387.
[4] *J. Physiol.*, 1907–8, **36** ; *J. Roy. Army Med. Corps*, 1908, **10**, 25.
[5] *J. Roy. Army Med. Corps*, Aug., 1913.
[6] A man regards fatigue products as greatly conducive to heat-stroke. *Compt. rend. Acad. d. Sci.*, 1917, **164**, 83.

In 18 cases the patient felt slightly unwell for a day or two before the attack.

In 21 cases the blood was examined for parasites of malaria; in 19 the result was negative.

In several cases it is mentioned there was complete absence of sweating.

Of drinkers there were 7 cases and 4 deaths; moderate drinkers 7 and 2; non-drinkers 7 and 1.

The body temperature rose above 106° F. in 33 cases, above 108° F. in 15 cases, and 9 of these died.

Cold-water douches and ice-water enemata gave excellent results, while antipyretic drugs were tried without success.

Out of 353 cases of heat-stroke which occurred at Amara, F. E. Fremantle [1] reports 64 as having a history of malaria, and 122 with a history of constipation. The latter is a sign of loss of tone, and thus heralds the failure of the heat-regulating mechanism.

At the time of attack 117 were reported as being on duty, 33 off duty, 108 as resting.

Deaths in cases with temperatures over 105° were distributed thus:

Body temperature	Cases	Deaths	
105°–106° F.	18	3	1 in 6
106°–107°	23	2	1 in 11·5
107°–108°	26	4	1 in 6·5
108°–109°	26	6	1 in 4·3
109°–110°	14	5	1 in 2·8
110°–111°	9	3	1 in 3
111°	1	–	
	117	23	

From his experience in Mesopotamia G. K. Hearne tells me that in two troop-ships from England, and free from malaria, there occurred several cases of heat-stroke on reaching Basra. 107°–108° F. seems to be the critical rectal temperatures at which grave pathological effects such as unconsciousness and convulsions first show when a patient is developing heat-stroke. Reduction from such temperatures must be carried out in the shortest possible time. If the treatment be at all delayed tissue degenerations occur and permanent injury may result even if death be escaped.

Men vary in the capacity of their sweating mechanism. In the summer of 1917 in Mesopotamia the temperature rose in stages, and groups of cases occurred at 105°, 110°, 115°, 120° F. One man may be refreshed by drinking a cup of hot tea when the temperature does not exceed 105° F., his sweating mechanism being then maximal, and above this must eschew tea and have iced drinks. Another man can be refreshed by tea when the temperature is 120° F.

Treatment of heat-stroke. The simplest means, if water is handy, e. g. in the case of stokers overcome in engine-rooms of ships, is to lay them on deck and throw pails of water over them, and fan them if there is a following wind and little ventilation.

Artificial sweating by wetting the skin and fanning it is the

[1] *Lancet*, Sept. 13, 1919.

obvious method based on physical and physiological facts, but these facts were unfortunately not generalized.

'The method of treatment of heat-stroke by fans and water has been,' writes General T. H. J. Goodwin to me, 'to the best of my belief, known and extensively practised in India for the last twenty years, and this method has been used, most certainly by myself and, I believe, many hundreds of others, in the earliest possible stage. It is, I think, generally recognized that the earliest signs of heat-stroke are suppression of sweating, and a more or less raised body temperature.'

In 'Memoranda on some diseases in the Mediterranean War Area', a reference book used by medical officers, it is stated, 'where ice can be got it must be used to bring down the temperature . . . where ice cannot be got a sheet soaked in dilute alcohol over which a draught of air plays may be tried or cold water enemata given'.

In 1917 the orthodox treatment seems to have been venesection, infusion, rubbing with ice, and copious ice enemata.[1]

While evaporation of water at body temperature carries away 590 gramme calories per gramme, the melting of ice only takes away 80 gramme calories per gramme; rubbing with ice obstructs the freedom of evaporation, and may constrict the capillaries, and its efficacy cannot be compared with that of wind used to accelerate evaporation. There is no need to use alcohol if water and fans are available, nor is iced water necessary, as the difference in total calories lost by evaporation is small between warm and iced water.

Ice-water enemata destroy the one reliable means of ascertaining the body temperature, and have nothing like the efficacy of cooling by evaporation. 70 grm. evaporated from the skin would take away as much heat as 1,000 grm. of iced water used as an enema.

The summer of 1917 in Mesopotamia was a terrible one; for three months the dry-bulb maximum ranged from 92°–121° F., from July 7–25 the heat was intense, and the maximum ranged from 110°–121° F., from August 16–Sept. 13 it ranged from 108°–121° F. This meant 130° or so in a tent on the hottest days.

Fremantle says the wet bulb failed to give any useful suggestion of dangerous conditions, and asks for the regular use of the kata-thermometer in future.

G. K. Hearne, from the experience of 226 cases which he treated in Basra in 1917, concluded that hyperpyrexial heat-stroke, uncomplicated by malaria or other high fever, cannot occur under natural atmospheric conditions, in the resting subject at least, unless preceded by suppression of sweating. He observed patients with suppressed sweating rise to a temperature of about 106° F., administered to them diaphoretics without a trace of sweat resulting, and then produced complete relief by applying to the patients the wet sheet and fan treatment. He thus proved that the giving of diaphoretics was useless and dangerously wasted time which should be spent on the application of the wet sheet and wind. After reduction of hyperpyrexia by these means (artificial sweating), when the patient is dry and settled in bed, the injection of $\frac{1}{5}$ gr. pilocarpine produced copious salivation but

[1] Milner, *Brit. M. J.*, Aug. 6, 1918.

no sweating—the exhaustion of the sweating mechanism still remained, an exhaustion signalized not only by the hot, burning, dry skin but by the increased frequency of micturition. He thus describes his initiation of proper preventive measures:

'One terrible afternoon, while hoping for a result from diaphoretics in the case of eight patients who had stopped sweating, three of these suddenly and almost at once became heat-stricken; the nursing staff divided their attention between two, and the third man was helped by some convalescent patients. He died in a quarter of an hour. The whole ward was in chaos, and patients with "the wind up" begged to be sent away from the awful climate.' The other two being relieved, Hearne fetched some flexible cord from the electric light station a quarter of a mile away, and fitted up hand fans by which he saved the others. Next morning he introduced the preventive system of fan and spray, and applied it to all who stopped sweating before heat-stroke ensued. The ward was peace and quiet for the rest of the season. This treatment became generalized.

Pembrey has pointed out that suppression of sweating occurs at the onset of many fevers, due probably to the toxin in circulation. Once the balance of heat regulation is upset by high external temperature and exhaustion of the sweat mechanisms a vicious circle is established, for the rise of body temperature increases the rate of metabolism.[1] Hearne found that suppression of sweating starts before there is any sign of vasomotor control giving way. There is nothing remarkable, too, about the diameter of the pupil. The attack results, then, not from a general exhaustion of the sympathetic system, but of the sweat mechanism.

The pulse is quickened but strong during the time immediately preceding the attack, and remains so till about ten minutes or so after cooling treatment has been started, when it becomes decidedly softer. In cases that have been left a long time before treatment this softening is also noticeable, but these patients are then practically moribund. At the start of the attack the skin is flushed, the general congestion suggesting that the over-heated blood is lying in all the large venous trunks of the body.

Hearne had observed that in the hospital huts it was a common occurrence for a patient to ask for the roof punkha above him to be switched off, the patient asserting that hot currents of air were driven down from immediately below the iron roof. On examining the patient, however, it was found that he had either completely or nearly stopped sweating, and, later on, a request of this nature came to be regarded as a sign that the patient required watching. Those who were sweating objected strongly to the punkhas being stopped.

Hearne made the following observations:

			Dry bulb	Wet bulb	Wet kata Heat loss	Dry kata Heat gain
Experiment 19	Punkhas turned off		109·2° F.	83·1° F.	6·37	2·17
,, 20	,, ,,	on	108	82·4	15·16	5·1
,, 27	,, ,,	off	109	80·6	10·58	3·03
,, 28	,, ,,	on	109	78·9	20·03	5·96

[1] Sutton, *J. Path. and Bacteriol.*, 1909, **13**, 68; Krehl and Marchand, *Allgem. Pathol.*, 1908, **1**, 104.

These experiments showed that in a patient who was sweating the current of air from the punkha increased his rate of heat-loss, 2 to $2\frac{1}{2}$ times in the case of the wet kata ; but in a patient with suppressed sweating the punkha was responsible for a *gain* of heat, by 2 to $2\frac{1}{2}$ times in the case of the dry kata.

The kata-thermometer showed that the punkha is a safeguard against heat-stroke in the one case, and a dangerous means of hastening its onset in the other. The obvious remedy of course is not to switch the punkha off but to put a wet sheet over the patient.

Hearne found the method of rubbing the body with ice is less efficacious than treatment with cold water and fans. The comparative advantage is very noticeable during treatment, the average time taken by the evaporative method being 20 to 25 minutes, while, by the use of ice alone, the reduction often takes three-quarters of an hour. In one case the temperature was lowered from 109·2° F. to 104·8° F. in 15 minutes and consciousness returned. The difference was illustrated by measurement with the kata-thermometer, experiments showing that the heat-loss due to evaporation was almost twice as rapid as with rubbing the kata-thermometer with ice.

In the first boat-load of troops that came up-river the state of affairs was described by Hearne as ' appalling '. The rest got off without a single death, and, although there was in each an average of 3 or 4 heat-stroke cases and 30–40 cases for preventive purposes under fan and spray treatment, this was carried out in perfect order.

The cooling room in the base hospital was provided with a waterproof floor, and hoses, with fine sprinklers attached, led from pipes placed over each bedstead. A tank in the room above fed these pipes, the water being kept by means of ice at 50° to 60° F. The walls of the tank and the pipes were jacketed. Each bedstead had a powerful electric fan beside it, so as to cool the patient by evaporation. For mild cases two bed cradles, covered by a wet sheet and fanned, formed a cool chamber.

If the patient had an enlarged spleen or previous history of malaria, quinine was given.

W. H. Wilcox writes to me that he has no doubt that the symptoms of heat-stroke are due to an auto-intoxication consequent on the effect of heat on the body cells. Many of the symptoms of prolonged pyrexia without recurring convulsions, &c., in the absence of bacteriological evidence of infection could only be explained in this way. In some cases there were symptoms of auto-intoxication, but no fever for several days, and then hyperpyrexia occurred. Excess of indican was common ; in a very few cases acetone and diacetic acid were present in the urine.

Hearne sees no reason why toxins should play an important part in heat-stroke, except in the later stages after the man has lain unconscious without reduction of hyperpyrexia for say $\frac{1}{4}$ to $\frac{1}{2}$ hour. He has seen in Mesopotamia treatment by venesection and transudation of fluid tried in numerous cases, but is certain the water spray and wind treatment would have pulled these cases through. Venesection should only be used for plethoric venous engorgement, and injection of gum saline for cases of collapse. Quick, shallow breathing washes out CO_2

from the blood and increases the alkalinity of the tissues. Injections of sodium bicarbonate solutions are then contra-indicated.

In the final stage of heat-stroke the patient lies cyanosed and comatose, with signs of respiratory embarrassment. *Post mortem* the organs always show vascular engorgement; fluid is transferred from the tissue cells to the dilated capillary vessels; a slice of the knife causes copious oozing of blood from the cut surface.

The lungs are very congested. Nissl granules are almost absent from the nerve cells. The adrenals and thyroid probably share in the congestion; if so, increased heat production may result from the activity of the thyroid-adrenal mechanism and contribute to the heat-stroke. The exhaustion of the sweating mechanism may not be the sole cause. Bacterial infections, by causing hyperadrenalism and pyrexia, contribute to heat-stroke, according to the view of Cramer.[1]

'It should be possible', writes Cramer to me, 'by post-mortem examinations to exclude an increased heat-production resulting from an increased activity of the sympathetic system. There ought then not to be a congestion of the thyroid, adrenals, and lungs. Congestion of the lungs has been observed. But pathologists never seem to look at the thyroid and adrenal glands.'

In mice, Cramer finds, stimulation of the sympathetic system combined with insufficient cooling will produce a heat-stroke. Thus a small dose of tetrahydro-β-naphthylamine will not kill a mouse kept in a cool room by itself, but will kill if the mouse is kept in a warm room, or if a number of mice are kept together in a small cage. Narcotics such as chloroform and chloral counteract this kind of experimental heat-stroke, but obviously cannot prevent the rise of temperature in a man who has ceased to sweat and is exposed to air-temperature above blood heat.

Tetrahydro-β-naphthylamine dilates the pupil, constricts the blood-vessels, causes rapid respiration, restlessness, greatly increased muscular activity, and spasms. There is an increased heat-production shown by a greater O_2 use and CO_2 output and due to the muscular activity.[2]

The drug causes intense congestion of the thyroid with changes in the colloid, very active secretion of adrenalin, disappearance of glycogen from the liver, and intense congestion of the lungs as if an overdose of adrenalin had been given.

Observations on lower organisms show that death from excessive heat is neither due to coagulation of proteins nor to want of oxygen, for the change produced in the living substance by heat is not affected by increased oxygen tension, while it is reversible if not carried too far. Thus if the pulsating subumbrella ring of a scypho-medusa is deprived of sense organs, an entrapped neurogenic contractive wave continues to run round it. The rate of the wave increases up to 37·3° C., and then declines. After being taken from water at 37·8° C, and put in water at 29·3° C., the rate at first remains less but recovers in two hours' time.

A. G. Mayer suggests that acid accumulates from the rate of metabolism being enhanced by heat, and this poisons the protoplasm.

Blackman for plants and Harvey for animals have advanced the

[1] *Sci. Rep. Imp. Cancer Res. Fund*, 1919, **6**, 1.
[2] Mutch and Pembrey, *J. Physiol.*, 1911, **43**, 109.

theory that some essential enzyme may be destroyed by excessive heat.[1]

It is to be expected that heat *per se* would disturb the delicate balance of the reactions on which the manifestations of life depend. The ultra-microscopic method shows an augmentation in the number of granules in living protoplasm, and a growth in the size of the granules at first visible, as the temperature rises.[2]

In general, vital processes become hampered by temperatures above 40° C., while below zero the water of the aqueous solutions in the cell freezes. The influence of temperature, between these limits, on the velocity of different processes in which enzymes and living cells are involved follows in many cases the same law as is found for the influence of temperature on chemical reactions.[3]

Bacteria live in hot springs, and certain unicellular organisms can be trained to survive temperatures far beyond the usual lethal limit, e. g. 60–70° C.

Rotifers when dried withstand 62·5° C., when in water and active 44·5° C. (Spallanzani).

Corals which live in cool, relatively agitated water free from silt cannot withstand the high temperatures which corals can endure that live in hot, silt-laden shallows near shore. The death temperature thus varies from 34·7° to 38·2° C.

Tropical lizards, together with alligators and certain aquatic reptiles and amphibians, easily support a high body temperature and are not exhausted by the consequent high rate of metabolism. On the other hand, the vital processes of a green lizard and frogs at temperatures above 30–35° C. become pathological, while temperatures above 25° C. are lethal to most marine animals which escape from sunlit pools to shady cool places.

[1] See A. G. Mayer, *Am. J. Physiol.*, 1917, **44**, 581.

[2] G. Matisse, *Action de la Chaleur et du Froid*, Paris, 1919, 383.

[3] Arrhenius, *Quantit. Laws of Biol. Chemistry*, London, 1915. See also Matisse, *cit. supra.*

CHAPTER XI

Food, Exercise, and Climate.

In successfully meeting the change from a temperate to a tropical climate, the regulation of the diet is of no little importance. The products of bacterial decomposition which arise in the bowel from overfeeding, ill-chosen food, or constipation, no less than a toxin arising from some infection, disturb the heat-regulating mechanism and lower the resistance of the body to hot atmospheres.

The 'specific dynamic energy' of the food-stuffs has also to be considered (Rubner).

If a fasting animal be placed in an environment which reduces its heat production to a minimum, e. g. a chamber at 33° C., and be given protein to the value of 100 calories it increases the basal heat production to the value of 131 ; the figure for fat is 113, for carbohydrate 106. When, then, either the environment is such that heat-loss is impeded, or the work so great that the body is in danger of overheating, carbohydrate is a better source of energy than protein.

In the fed, and particularly in the over-fed, production of heat is enforced by the high specific dynamic energy of protein, the increase of heat production over the fasting value growing with, and nearly in proportion to, the amount eaten. While the influx of carbohydrate, of fat, or of alcohol enables the cells to oxidize at a higher level, through the increased mass action of food particles which are available, the metabolism products of protein, the amino-acids, directly stimulate protoplasm without themselves being involved in the oxidative process ; thus 20 grm. glycocoll (fuel value 42 calories) increased the basal metabolism of a curarized dog 33·7 per cent., 10 grm. half this amount, the increase being proportionate to the quantity fed (Lusk).

Protein does not exert its specific dynamic action when eaten in conditions of under-nutrition, but spares an isodynamic quantity of body substance ; so, too, in respect of protein used for growth, so long as no more is ingested than the heat requirement of the body calls for. The following figures, taken from Rubner—I have added dry kata-thermometer cooling powers for the still air of the calorimeter—illustrate how a meal of meat almost doubles the caloric output in the dog in a warm atmosphere.

		Caloric output		
Temperature of air	Dry kata	Fasting	550 grm. of meat	Per cent. increase
4·2° C	8·7	128·1	133·5	4·2
14·5	5·9	100·9	110·9	9·9
22·1	3·9	70·7	101·0	42·9
30·7	1·3	62·0	117·2	89·0

Rubner[1] also gives the following figures for the dog :

Excess of protein eaten above requirement	Increase in heat production
56 per cent.	19 per cent.
90	35
105	44
158	49

[1] *Die Gesetze des Energieverbrauchs*, Berlin, 1902, 246.

These are increases in total metabolism comparable to those induced by a considerable amount of physical work: obviously, then, a heavy meat meal lessens efficiency in and increases the danger of a hot environment; after a meal of meat a dog's heat production is not increased by exposure to cold as a fasting dog's is, since the fed dog has a heat production in excess of the requirement. All that is necessary in it is regulation of heat-loss by more, or less, exposure of the body, dilatation of cutaneous blood-vessels, and evaporation of water.

The unheating effect of carbohydrate food compared with protein is shown by the following figures given by Rubner for man.

Air 20° C. Relative humidity 40 per cent.

	Total output in Calories	Per cent. increase	Calories due to work	Water evaporated Calories	Loss by radiation and convection Calories	Calorie value of work
No food, rest	1976	—	—	380	1596	—
Cane sugar 600 grm. + 3000 grm. water, rest	2023	2·4	—	529	1494	—
Ditto + 100,000 kg.,work	2868	45·2	845	907	1727	334
Large amount of meat eaten, rest	2515	27·2	—	614	1901	—
Ditto + 100,000 kg.,work	3370	70·5	855	1235	1901	334

The work was done more easily on the cane sugar than on the meat diet.

The figures show that under the atmospheric conditions of the experiment the increased heat production due to the specific dynamic action of the meat protein added itself to the increased heat produced by work which had to be sweated off. In the case of the cane sugar diet only half the heat produced by the work had to be sweated off, because the sugar was almost completely utilized for work and had almost none of the specific heat-producing action which protein, or its amino-acid derivatives, have. Protein has, however, no wasteful action on a subject working in an environment where the cooling power is very great and the heat generated by work insufficient to maintain the body temperature. Hence more meat is naturally consumed by those exposed to hard climatic conditions. The man on a sailing fishing-smack needs far more than, say, an R.N. officer on a battle-ship, in the close confinement of the steel walls of which much of his time is spent.

The temptation for over-indulgence of the appetite on the part of the latter, and no less of officers in the army, is considerable.

The ordinary diet of an officer on a battle-ship, living a life largely in confined quarters and with little hard exercise, is something like this:

8 a.m. Breakfast: fish; bacon and egg; toast, butter, and jam.
11 a.m. Glass of wine and biscuit.
12 noon. Three-course lunch; glass of beer or whisky.
4 p.m. Tea; bread and butter.
7 p.m. Dinner: soup, fish, entrée, joint, sweet, cheese, wine or whisky.
10 p.m. Cocoa and biscuit.
4 a.m. Cocoa and biscuit.

One course could with advantage be knocked off breakfast, lunch, and dinner, and the 11 a.m. and 10 p.m. snacks abolished. The officer does get exposure on deck, or on the bridge, while the city business man who indulges no less often gets almost none.

In tropical climates fruits, salad, green vegetables, boiled rice, and a little fish or bird and dairy produce form a suitable diet for hot weather. The self-indulgent man should secure a comfortable feeling of repletion by eating more watery foods, e.g. fruit and green vegetables with 85–95 per cent. of water, and reducing such concentrated foods as sugar containing no water, butter 10 per cent. water, and bread 35 per cent. water. The necessary vitamines and amino-acids can be secured from a diet of fruit, eggs, green vegetables, and milk puddings with least energy value and lowest specific dynamic action.

The obese work under great disadvantages in a hot moist climate.

The following shows the effect of work, temperature, and humidity in a chamber on a fat man's metabolism.[1]

Light work 5,375 kg. per hour.

	Dry air		Humid air	
	CO_2 grm. per hour	H_2O grm. evaporated per hour	CO_2 grm. per hour	H_2O grm. evaporated per hour
20°	47·8		46·4	
28–30°	47·3		48·0	
36–37°	50·3	319 + 38 sweat	60·7	269 + 266 sweat

The cooling effect of the sweat was not sufficient, and the small amount of work was accomplished at 37° at the expense of great discomfort and profuse perspiration, the rate of metabolism rising owing to increase of body temperature.

Rubner found a lightly-clad fat man bear a temperature of 30° with humidity 65 per cent. so badly that he feared to raise the temperature to 35°. Such conditions are far better borne when the body is thin and the diet of low protein value.

Europeans become thin in the tropics, and thin men of light weight appear to be most suitable for tropical posts.

In the case of a man 6 ft. 2 in. in height, w' ose weight through war rationing sank from 163 lb. to 101 lb., the basal metabolism diminished from 40 to 25·4 calories per sq. m.

In the tropics light weight has then a particular advantage. The difference pictured between John Bull and Uncle Sam is a climatic one.

Heavy-weights are uneconomical people, for they each have to feed a large mass of living cells.

Benedict found, in the case of a squad of college men whose average net calorie use was 3,100, that after rationing and an average loss of 12 per cent. of body weight 1,950 calories sufficed and the normal amount of work continued to be done. Their heat production per sq. m. of body surface decreased 22 per cent. There was greater economy of muscular effort. The nitrogen output was lessened from 14 to 10½ grm. a day. The pulse-rate and blood-pressure were lowered. The men were chilly and their sexual desire in abeyance.

Magnus Levy [2] reported the case of a neurasthenic youth who had partially starved himself for a year. With 30 per cent. reduction in weight from the normal, the metabolism per sq. m. of surface was reduced on the low dietary 33 per cent. below normal, on a liberal diet 17 per cent.

This is an extreme example of how the sedentary indoor life and

[1] Broden and Wolpert, *Arch. f. Hyg.*, 1901, **39**, 298, cit. after Lusk.
[2] *Ztschr. f. klin. Med.*, 1906, **60**, 177, cit. after Lusk.

strain of the system of education and city life with its sexual difficulties provokes neurasthenia and reduces metabolism. So, too, the tropics may strain a white man. When health begins to decline the sensations are blunted and, the feeling of hunger being feebly experienced, no great efforts are made to satisfy it.

In England, owing to prosperity, over-eating has become habitual with many. A young man, poor, keen, active, and frugal, when he gets prosperous will overload his stomach three or four times a day. He will eat freely of every course, any one of which would have sufficed for his meal in the early days of struggle, and this, too, when he is old and indolent and needs far less food.

The natural inclination of man, when well-to-do and engaged in sedentary occupation, is to raise his metabolism rate by increasing the protein value of the diet. He takes also alcohol, or hot stimulating drinks—tea, coffee, meat extract. He enjoys a warm skin, and thus replaces the natural increase of metabolism and warmth of skin which is produced by open-air exercise. Hot food, iced food in hot weather, alcohol, coffee, tobacco, take the place of the natural stimulant, the ever-changing condition of open air. The over-indulgent man who floods his body with food and alcoholic drink (alcohol is, so to speak, predigested, ready for metabolism, and is therefore first used in the body-furnace) has then to get rid of the surplus heat produced, and if he is living an indoor life and over-clothed, develops an extra radiation in his naked parts—the flushed face of the diner-out, the dilated venules in the purple cheeks and nose of the chronic *bon vivant*.

No such development takes place in the kilted ghilly on the Scottish hills, who may even drink a bottle of whisky a day with impunity, for the cooling power on the hills is such that the heat is lost from the skin as fast as produced, the alcohol acts but as fuel to keep up the normal body heat. So with the troops exposed to mud, water, and the cold night mists of the trenches. To them the rum ration was a necessity, a quick-burning fuel keeping up the body heat, and supplying energy to the tired heart. There was no means of replacing the rum by substitutes, such as hot soup or hot sweetened coffee or tea.

So with workers in hot furnace rooms; they need a food easily assimilable, supporting the heart strained by the double call upon its energy for work and for cooling, a food which does not force up the heat production as protein does. For these men beer appears to be both food and drink. As a food it contains neither vitamines[1] nor the necessary proteins. Thus exposure to excessive heat leads to food deficiency. The over-fed man of means takes rides in fast motor-cars and thus secures a high cooling power to relieve him of the heat generated by his meat and drink. Thus uselessly and most uneconomically is the energy of food and petrol wasted on the self-indulgent.

More protein is required and consumed in cold climates; in the arctic expeditions flesh and fat of penguin, bear, and seal have formed the diet of parties cut off from their ships and isolated for months, e.g. in Nansen's, Scott's, and Shackleton's expeditions.

The sedentary brain-worker in temperate climates who takes no

[1] Beer made from germinated grains contains water-soluble *A* vitamine. Harden.

vigorous exercise suffers from deficient heat-production. He needs, therefore, a diet of easily digested food, small in energy value, e.g. 2,200–2,600 calories, and containing enough protein to keep him warm by its specific dynamic action. If carbohydrate in excess of need be eaten it undergoes bacterial fermentation in the bowels, and produces disturbances of health. The sedentary worker with depressed appetite feels chilly, puts on more clothing, and has lessened sexual power.

Most European and American diets provide more than 10 per cent. of the energy value in the form of protein.

It must be borne in mind that the various proteins are of very different value for human nutrition. A man can, it is said, be brought into equilibrium with a diet containing 25–35 grammes of protein of animal origin, 39 grammes of potato origin, and 84 grammes of bread origin. The dictum, as expressed by Bayliss, has been generally accepted, 'Take care of the calories and the protein will take care of itself'; and this is true if the diet is widely chosen and varied enough. On restricted diets the biological test of feeding can alone determine whether the food accessory bodies, amino-acids, salts, &c., suffice for growth and health.

Thus McCollum found that a cow fed on the whole of the corn plant, green leaves, stalk, and seed, flourished and bore a fine healthy calf, while another cow, fed on the stalk and seed of wheat, suffered severely from malnutrition, and bore a still-born calf.

Adequate growth has never been obtained with rations in which the nitrogenous components fail to furnish sufficient preparation of tryptophane, lysine, and cystine.[1]

If, then, the intake of protein is kept low, or there be not enough variety of protein food, a plane ultimately may be reached where the yield of the amino-acids in question becomes so small that it cannot satisfy first the growth requirement, and later the maintenance of certain physiological functions.

Chittenden has rightly urged the reduction of the protein excess which is eaten by many sedentary workers, but this reduction must not be carried too far. In strenuous physical workers a high amount of living muscular tissue requires to be maintained. The brain-worker who keeps his body fit by physical exercise therefore eats more, without over-indulgence, and secures a wider choice from which his body may select the subtle principles required for virile executive and imaginative work.

The races that eat animal protein in India are far the most virile. Eurasians, eating both more protein and more of animal origin than Bengalis, put on more weight, increased more in chest measurement, and were superior in athletic tests (McCay).[2]

The Bengali diet may be deficient in food-accessory bodies rather than in protein, or deficient in both.

The habit of lumberers, &c., in cold climates, of consuming much meat is directed towards securing fat rather than protein. Carbohydrate is much more rapidly digested and utilized than fat, and fat comes into use in the later hours of the working shift.

[1] E. G. Willcock and F. G. Hopkins, *J. Physiol.*, 1906, 35, 88.
[2] *The Protein Element in Nutrition*, London, 1912, 138 and 160.

The Japanese soldier is content with 20 grammes of fat a day, and satisfies 80 per cent. of his energy needs with rice, fish, and salads. It is important to note that the Japanese workers take salads all the year round as an essential article of their diet, and so secure both the water-soluble and fat-soluble food-accessory bodies.

The South Italian peasant in his home probably takes less than 60 grammes of fat a day. He eats corn-meal, olive-oil, and green stuffs. Seventy-five grammes is considered desirable in this country, or one-quarter of the energy value of the diet as fat. In America, where there is abundance of animal food, as much as 35 per cent. of the energy value may be taken as fat.

A Japanese jinricksha man can drag a heavy man 30 miles a day at a running pace, and keep it up for three weeks. According to Hindhede very hard muscular work can be kept up and health and long life secured on a diet of potatoes, fat, and milk. A diet of milk, porridge, fish, potatoes, and barley bread has conduced to the long life of the Hebrides peasants. McCollum connects virility of race with the consumption of dairy produce.

Denmark during the war reduced the stock of pigs to 17 per cent., and cattle to 65 per cent. of the pre-war average, giving the grain and potatoes so saved to the people. There was scarcity of meat in the cities, but no starvation, and, apart from the influenza epidemic, good health. Germany, following the opposite plan, came to disaster.[1]

The influence of open-air exercise upon the course of protein metabolism is of great significance.

The preliminary cleavage of protein into dextrose and nitrogen-containing residue, while yielding heat to the body, does not increase the vital activity of the cells except in so far as the heat set free affects the velocity of reaction in the liver, where the decomposition takes place. The muscular activity is ruled by the nervous system, and, if reduced to a low level in the sedentary, the dextrose, &c., set free from the decomposition of excess of protein eaten may act on the body to its disadvantage.[2]

Lusk finds 52·5 per cent. of the total energy of protein may be available as sugar, while 28·5 per cent. is set free as heat during the cleavage into amino bodies and separation of nitrogen from these.

E. P. Cathcart and J. B. Leathes[3] showed the effect on the endogenous formation of uric acid of (1) the reaction of the body to severe chilling, (2) muscular work. The subject lived on a purin-free diet of bread, butter, and milk. To secure the chilling effect he spent $2\frac{3}{4}$ hours naked in a swimming bath (water 71° F., air 64° F.), standing about most of the time and entering the water whenever the skin dried. He shivered at times. His rectal temperature, which was 100·8° F. on reaching the bath after a 10 minutes' walk, fell to 97·7° F. As a result he notably increased his excretion of uric acid, the reaction lasting over three days.

Voluntary muscular exercise, on the other hand, diminished very notably the production of uric acid. In the performance of work during a corresponding period of $2\frac{3}{4}$ hours the subject wore winter

[1] Hindhede, *Ugesk. for Læger*, 1919, **81**, 183.
[2] Sugar is increased in the blood in the tropics. See p. 96, note 2.
[3] *Proc. Roy. Soc.*, 1907, **B. 79**, p. 541.

clothing, and a thick knitted jersey in addition. He walked 2½ miles in half an hour, worked a heavy floor rubber about for half an hour, then repeated the walk and the work with the floor rubber. His rectal temperature had risen from 99·8° F. to 102·4° F. after the walk, and over 102° F. at the end of the period of exercise.

Here, then, we have notable evidence of the influence of chilling exciting, and exercise restraining the endogenous formation and excretion of uric acid. The problem of establishing a causal connexion between uric acid and the etiology of gout has hitherto remained unsolved, but it is an established experience that ailments connected with over-indulgence in food can be cured by vigorous open-air exercise no less than by moderation in food. Gouty people also are often upset by east winds or exposure to a bracing climate, to high cooling and drying powers, without hard exercise.

An analysis of the cases of arterio-sclerosis and fibrosis in well-to-do people who were treated at a hydropathic institution showed that almost all the patients had lived sedentary indoor lives, many took practically no exercise, few took baths, most were over-indulgent in food.

Of 150 cases of very long-lived persons analysed by Sir Hermann Weber,[1] by far the majority were temperate, small meat-eaters, lived much in the open air, led an active life full of work, many a life of toil with great restrictions as to food and comforts, most were early risers and worked cheerfully. Only a few were self-indulgent or intemperate, or idle and lazy persons.

Weber ordered the life of middle-aged men whose family history for longevity was bad and whose health was failing, putting them on a regimen of restricted diet with great moderation in meat and alcohol, and daily vigorous open-air exercise, and thus extended their lives to some eighty years, while their brothers and sisters put on no such regimen died at some sixty years. There can be no doubt that moderation in eating and drinking, and vigorous open-air exercise taken daily, keep off the degenerations and pains and aches of old age, which are started either by toxins arising from infections, or bacterial fermentations in the bowel, or by the products of a deranged metabolism, e. g. lack of vitamines from ill-chosen diet, or waste products which arise from or accumulate in the tissues through want of oxygen.

To number some of the beneficial effects of exercise—it promotes increased blood-flow in and supply of oxygen to all parts of the body, an increased fluid exchange between the tissues and blood, and facilitation of the removal of waste products. It promotes the metabolism and production of body heat, massages the belly organs, and causes the better absorption and utilization of the food, so that the bowels are kept open and free from excessive bacterial decomposition and toxic products arising therefrom. Thus the resisting powers of the whole body to disease are kept up, the brain is swept with a fast flow of well-oxygenated blood, and the mental faculties kept in good condition; the lungs are benefited by the deep breathing resulting from exercise, the ample pulmonary circulation, the evaporation from and flow of lymph through the respiratory membrane; the skin by the enhanced circulation, the evaporation from and flow of lymph through it. The circulation of the blood being aided by exercise,

[1] *Longevity and Prolongation of Life*, 5th edit., London, 1919.

freedom of joints and bones is ensured from toxic products of digestion or infection, the blood-forming organ, the red marrow, and the blood being benefited and immunity to infection kept up.

The magnificent complexion and health, the absence of nerves, and greater enjoyment of life are most marked in girls who have worked on the land compared with girl typists or the chorus girls of a theatre.

The effect of active exercise and of a sedentary life on the bodily organs of white rats has been studied by Hatai.[1] He kept rats during the daytime in revolving cages which forced them to take continual exercise. After 3–6 months the animals were killed and compared with those which had led a quiet life. The heart, kidney, and liver of the exercised rats were 20 per cent. heavier; the testes 12 per cent., the ovaries 84 per cent. The spleen and thyroid were decreased in weight, the suprarenals unaltered in the male, but 48 per cent. heavier in the female; the pituitary body increased 10 per cent. in the male and decreased 22 per cent. in the female.

Semi-starvation lowers the bodily resistance to all infections: the type of a famine sickness will depend upon the prevailing epidemics. Prevalence of boils and skin affections signalize the weakened resistance. Cold and labour notably hasten the decline of health in those who are semi-starved.

Deficiency of food-accessory bodies and essential amino-acids is probably much more potent a cause of the lowered resistance than lack of energy value. A deficiency of the relatively large intake of calcium and phosphoric acid also promptly leads in interrupted growth and nutritive decline, while surprisingly small quantities of chlorine, sodium potassium, and magnesium suffice.[2]

Osborne and Mendel have shown that white rats can be kept alive, while stunted in growth, by a suitably chosen diet deficient in energy value, and that after months of this they make up full growth and sexual power when amply fed. Breeding-power can thus be put off to a later date than normal, and duration of life perhaps prolonged. (Fig. 28, p. 197.)

Greenwood has discussed a reduction of the dietary of prisoners at Millbank[3] in 1816 to approximately 2,500 calories, which was followed by debility and epidemics of scurvy and dysentery. The diet consisted of bread, soup with some vegetables boiled in it, and hot gruel or porridge. Probably the essential factors were deficiency of fat-soluble *A* vitamine, and destruction of the water-soluble *A* (antiscorbutic) vitamine by cooking of the vegetables (very limited in quantity) in soup.

In contrast a diet at Devizes Jail of value 2,200 calories but containing 1 pound of potatoes daily sufficed to keep the prisoners healthy. These, be it noted, were not performing any hard labour.

He points out that in the siege of Paris the weather was cold and fuel as scarce as food. In the twenty-second week of the siege 627 persons died of bronchitis against 76 for the same week in the previous year. The death-rate from pneumonia was some five times the normal. Typhoid, dysentery and diarrhoea, and small-pox claimed many, the death-rate rising to 38·6 per 1,000 from 13·1 in the previous years.

[1] *Ann. Rec.*, 1915, **9**, 647.
[2] Osborne and Mendel, *Year Book Carnegie Institute*, 1918, 302.
[3] Greenwood, *Roy. Soc. Med.*, Epidemiol. Sect., 1918, **11**, 61.

In the siege of Kut-el-Amara the rapid decline of vigour and health corresponded with the reduction of the caloric value of the diet to about 2,000. There were many deaths from enteritis. The ration was first reduced on January 20. On April 10 it fell as low as 2,000 calories. On April 29 the surrender took place.

In both these cases there must have been deficiency of food-accessory bodies as well as low energy value.

The semi-starvation in the areas devastated by war has produced a vastly increased incidence in rickets, dysentery, tuberculosis, scurvy, together with lessened fertility and efficiency.

The want of food-accessory bodies stunts growth, causes digestive troubles, brings about decay of the teeth, malnutrition of the nervous system, atrophy of the muscles and bones, diminution of red blood corpuscles and the red marrow which forms them, atrophy of the sexual organs and loss of fertility, and renders the subject most liable to bacterial infections. While a scorbutic diet lessens the function of the adrenal glands, a diet deficient in vitamines causes degeneration of, and atrophy in, the coats of the bowels. Mucous and coeliac disease and intestinal stasis may have their origin in vitamine deficiency.[1]

Concerning these food-accessory bodies the reader may be reminded there are recognized[2]:

Water-soluble *A* substance, the want of which causes scurvy.

Water-soluble *B* substance, the want of which causes beri-beri.

Fat-soluble *A* substance, the want of which causes defective growth, rickets, decay of teeth, &c.

The food-accessory factors are present in growing cells, e. g. the green leaf of vegetables, glandular organs, liver, kidney, sweetbread, in the germs of seeds, and in eggs and milk feeds prepared for growth-maintenance.

The milk of the cow fed on spring grass is richer in fat-soluble *A* than the milk of the cow fed on artificial food and roots. So, too, the milk of the mother fed on food poor in food-accessory bodies is deficient, and the body fed on such will be under-nourished or suffer from malnutrition, e. g. rickets, from want of fat-soluble *A*.

The water-soluble *A* body is easily destroyed by cooking, and raw fruits and salads or vegetables boiled only for a short time are required in the diet to keep up its supply.[3] If milk is boiled or dry milk used orange juice should be added for the baby.

Water-soluble *B* is removed by the miller in the preparation of white flour and polished rice. The use of polished rice in the tropics has been the cause of beri-beri.

Fat-soluble *A* is not present in vegetable-oil margarine, and there is not much of it in ordinary butcher's meat.

The industrial worker's diet, then, of white bread, margarine, meat, sugar, and sweet (white flour) cakes and tea, while supplying ample energy, is deficient in food-accessory bodies.

Potatoes make good the water-soluble bodies, but milk or the milk

[1] McGarrison, *Indian J. M. Research.*, 1919, **6**, 275, **7**, 167, 188.

[2] Cf. F. G. Hopkins, *J. Physiol.*, 1912, **44**, 425; Osborne and Mendel, numerous papers in *J. Biolog. Chem. U.S.A.*; Harden, H. Chick and others, ditto, *Biochem. Ztschr.*; E. Mellanby, *Lancet*, Mar. 15, 1919; McCollum, *The Newer Knowledge of Nutrition*, New York, 1919. *Medical Research Committee, Special Report Series, No. 38*, 1919.

[3] It is present in germinated grain and in beer from such.

products, butter and cheese, and green vegetables are required as *protective foods* to ensure a sufficiency of fat-soluble *A*.

The depression of metabolism produced by sedentary occupation in stagnant atmospheres must act as a contributory cause of malnutrition and 'deficiency' diseases, when there is a lack of essential amino-acids and food-accessory bodies in the diet, as is commonly the case in cities.

The significance became apparent from the above considerations of (1) stimulating metabolism by exercise and cooling power of open air so that enough food is eaten to secure the growth principles; (2) garden cities in which a sufficiency of fresh green vegetables can be grown by the householders, and ample opportunities of open-air exercise obtained, (3) keeping up the supply of dairy produce to the highest level.

Effective measures for preventing rickets, decay of teeth, and tuberculosis are, then, a properly balanced diet and open-air exercise which creates the physiological demand for food and ensures its healthy digestion, absorption, and utilization.

The body of a strong young man contains some 18 per cent. of fat, and in a 70 kg. man this means a reserve of 11,700 Calories, a provision of 1,000 Calories a day for twelve days.

While there is an advantage in building up a normal reserve of fat against illness, there is no advantage in over-feeding consumptive patients and deranging the bowels. Heavy meals of meat and pastry-cake stuff seem specially uncalled for.

'Liberal consumption', says McCollum,[1] 'of all the essential constituents of a normal diet, prompt digestion and absorption, and prompt evacuation of the undigested residue from the intestine before extensive absorption of products of bacterial decomposition of proteins can take place, are the optimum conditions for the maintenance of vigour and the characteristics of youth.'

Ample milk and the green leaf vegetables in the diet and ample open-air exercise will secure these ends.

The good effect of prison diet and open-air exercise on sedentary over-fed men is a well-established fact. The wrong and the right treatment is exemplified by the following case:

A conscientious objector, a well-built, well-developed man, after a residence of three months at Wormwood Scrubs was seriously reduced in weight, strength, and vitality, owing to the close confinement and mental worry. A healthy, active man, he could not maintain a good appetite with little or no fresh air or physical exercise. The food, although plain, was in his opinion fairly good and sufficient in quantity. At the end of this time the prisoner was sent in frail and feeble state to Dartmoor. The active outdoor life, haymaking, &c., soon worked wonders, in spite of the somewhat meagre diet and the prison cell occupied by night.

At the end of six months he had gained 37 lb. in weight and had never felt stronger or more fit in his life. The work began at 7.30 a.m. At noon there was a break of an hour for lunch, and then work till 6 p.m. From 6.30 to 9 the prisoners were free to go walks or indulge in recreation. At 9.30 p.m. they retired to their cells. The work was never heavy or violent, but enough to keep them active and

[1] *The Newer Knowledge of Nutrition*, New York, 1919.

fit. According to this prisoner's report breakfast consisted of ¼ oz. margarine, 3 oz. bread (90 per cent. wholemeal), 1½ oz. plain oat cake, and a pint of cocoa without sugar or milk. Lunch—4 oz. of bread and 1 oz. cheese and a pint of cocoa. Dinner—2 potatoes (about 6 oz.), 1 oz. bread, 1 oz. cheese, a little cabbage or plain boiled rice or haricot beans or swedes, about 5 oz. of plain boiled margarine pudding with a little jam. There were 1,200 men there, and about half chose this vegetarian diet. Meat was substituted for cheese in the case of the others. No doubt this diet was amplified by the occasional purchase of food, for the prisoners were allowed to visit a village, but the frugal nature of the diet shows how important are absorption and digestion made perfect by conditions of life.

At the Harper-Adams Agricultural College, Salop, the effect of keeping cattle out of doors in the winter has been tried over a period of four years. Half the College herd were brought in at night and turned out on fine days only, half were kept out day and night, and only brought in for milking. The results were in favour of keeping the cows out—there was a greater increase in live weight, and both yield of milk and the butter-fat percentage in it supported the outdoor management. Cattle were also fed off crops grown on arable land while being kept in buildings so constructed that the animals practically were in the open air; these gave a much higher milk yield than grass-fed cattle, owing to the better feed off arable land.

T. B. Wood and A. V. Hill[1] measured the skin temperature of cows, and found that those with the lowest temperature did best and put on more fat. The good 'doers' on the average had a skin temperature 3° C. lower.

[1] *Journ. Agric. Sc.*, 1914, **6**, 252.

CHAPTER XII

Conclusions as to Colour of Skin and Climate.

1. The primary function of pigment is to arrest excess of sunlight which has a lethal effect. This is shown by the fact that (1) the blackest races are to be found in dry, sunny climates with much sun glare from the ground, (2) dwellers in the arctic snows are pigmented, (3) whites exposed to direct sunlight or snow glare and becoming sunburnt pigment, and after pigmentation become immune to sunburn.

2. The ultra-violet rays (between 3,200 and 2,300 A.U.) have a specifically powerful lethal effect on bioplasm, but are filtered out by the horny layer of the epidermis. The red-yellow rays alone penetrate in any quantity through the epidermis and dermis, as is shown by red glare seen through the cheek of a white man when a glow lamp is held in the mouth.

3. The visual rays, particularly those of the blue end of the spectrum, have a lethal effect on bioplasm if sufficiently powerful and prolonged in action.

4. Much of the sun rays are diffusively reflected by the scales of the scarf skin, still more by the film of moisture when the skin is wet with sweat. The practise of oiling the skin secures better diffusive reflection.

5. Arab horses and Indian cattle are protected from light by white or light-coloured short hair, which diffusively reflects, and a pigmented skin and panniculus adiposus, which absorbs the light that penetrates the hair.

6. Black skin round the orbits diminishes diffusive reflection into the eyes and so lessens sun glare. Artificial blackening has the same effect and is utilized by coolie mothers and mountain climbers.

7. Pigment placed in the deeper layers of the epidermis, by absorbing most of the visual rays which penetrate the horny layers, protect the blood and living tissues from the lethal effects of these rays.

8. The pigmented man can therefore have a much thinner horny layer and safely glow with flushed blood vessels, thus losing heat by radiation and convection, without risk of injurious effect from ground glare and sky shine, or from the direct sunlight, as he turns from shade to sun. A thin skin is important in promoting conduction, as the horny layer is an exceedingly bad conductor of heat.

9. The black skin absorbs far more sun rays than the white. But at body temperature the absorption and the emission of long radiations by the white and black skin is the same. The theory is erroneous which attributes the blackness of the negro to a higher emissive power suitable for a hot, shady, forest climate, and the whiteness of the white to a lower emissive power suitable for cold climates.

10. Any black surface is a trap for catching sun rays and converting

these into heat rays, i.e. short into long radiations. The black fur and skin of a piebald animal shows a higher surface temperature than the white fur or skin.

While it is an advantage to stop lethal rays by pigment, an extra tax is put on the heat-regulating mechanism in getting rid of the heat into which the light rays are converted by the pigment.

11. The plexiform nerve endings lie between the pigment cells and, excited by the sun energy converted into heat (or possibly into chemical energy) by the pigment, cause (1) the black man to take shelter, (2) his cutaneous vessels to dilate, (3) his sweat glands to sweat.

A black animal which cannot sweat is at a disadvantage in the sun, and if exposed to it requires frequent immersion in water.

12. The sweat protects from the sun's heat by virtue of (1) the 580 calories required for the evaporation of 1 grm. of water, (2) the glistening reflecting wet surface it gives to the skin, (3) the screen of water vapour it gives the steaming body.

13. As pigment renders the coloured man indifferent to sunburn, he can go naked and secure the full cooling power of the environment, with the heat lost by radiation, convection, and evaporation untrammelled by the confined atmosphere of house or clothes. He does, however, protect himself from the mid-day sun, and in America the negro wears clothes like the white man.

14. The white, in so far as he is afraid of sunburn, wears clothes from the point of view of caste and custom, and confines himself in shady, close places, is at a disadvantage from the point of view of heat loss. His customary diet, in particular the amount of protein consumed with its high specific dynamic energy, sets his heat production at too high a level.

15. When the white enters the tropics he should wear the loosest and lightest clothes and allow himself to become tanned, adjusting his diet and working hours to those of the natives, keeping up his metabolism by open-air exercise in the early morning hours, and sleeping away the heat of the day. He should use double-roofed shelters and conveyances, with free ventilation of the air space between the roofs. He should sleep on roofs exposed to the breeze, and use fans to work by. His clothes should be shorts and a white shirt with short wide sleeves and open at the neck and sides to allow free ventilation.

16. Custom and fashion should not forbid white women to secure in their dress sufficient exposure and ventilation of the body surface. They should freely take exercise, and not confine themselves or their children within houses, or strive to preserve their whiteness of skin.

17. Experience of wiser customs would show how far the greater disability of white women to live healthily in the tropics and their lack of fertility is due to their greater confinement by clothes and within houses, their want of exercise and lower rate of metabolism.

The climatic adaptation cannot depend merely on pigment, for a very moderate pigmentation, such as the yellow of the Mongol or the sunburn pigmentation of the white, suffices to prevent the lethal effect of the sun. Moreover, the pigmented Esquimaux is not fitted by his pigment to stand a tropical climate. The blackest races do not face the noon-day sun, but work in the early morning hours, and

secure the protection of shade in the day-time. Arabs, Hindus, &c., protect themselves with clothes from the sun.

18. There appears to be a correlation between the metabolism and heat-losing mechanism, which adjusts the race to the climate it has been evolved in. The kind of cattle found suitable for tropical climates, e.g. for draught oxen, have light hair and pigmented skins. Arab horses have pigmented skins. The native human races adapted to the tropics are pigmented. Pigment is then a sign of adaptation.

19. By the elimination of tropical parasitic insect- and water-born diseases the tropics can be made far more healthy for whites, and field labour in the tropics can be successfully endured by white men, but the women appear to age early and become barren.

It probably is the stagnant, moist environment produced by clothes and houses which tells against the metabolism and health of women. The men working stripped in the open keep up their metabolism and vigour. A more open life for women, the better construction of houses, and the wearing of clothes as suitable as those worn by men may prevent these ill effects.

20. The same comfortable, happy home life and enjoyment of field labour is not secured by whites in the tropics as in temperate climates.

To meet the conditions a virile fully-adapted race can no doubt be evolved slowly by natural selection of survivors, quickly by inter-breeding, e.g. a cross between Mongol or Amer-Indian and European is said to produce most virile types.

If tropical Australia is kept 'all white' by legal enactment, probably such cross-breeds who pass as whites and gain entry may be the survivors and their children come to people the land.

SECTION II

CHAPTER I

The Chemical Impurity of the Atmosphere in Crowded Confined Places and in Mines.

Before the discovery of the microbic origin of infective disease, the pioneers in sanitary reform associated the herding of human beings in confined and filthy quarters with the spread of pestilence, and attributed the cause of such to poisonous exhalations of the human body. It was observed that 'effluvia emanating from dead corrupted matter, even in a state of putrefaction, does not produce fever of an infectious type, and that, when these exhalations seem to engender such a disease, they consist in part, or are mixed with, the vitiated effluvia from the living human body; and that it seems to be an invariable result that the accumulation and stagnation of the breath and perspiration of human beings crowded for a period in confined air, and neglecting personal cleanliness, produce plague or fever, that may be communicated to healthy persons by contact or respiration.'[1] It was noted in England that the bubonic plague never became epidemic before the beginning of July, and reached its height in September, after which it declined. With a warm, moist atmosphere and the want of wind, 'the great ventilator of nature', every squalid inmate of an ill-ventilated apartment became a reeking sphere of pestilence, impregnating the motionless air of the dwelling. Erasmus, writing to Cardinal Wolsey's physician, pointed out how plague might be stopped. The houses, he said, were without any free ventilation, and were built without the least regard to this necessary condition of health. Windows were never opened, and no one cared to have a pure and wholesome air. The floors were beyond measure filthy, usually made of clay covered with rushes so little changed that the lower part remains sometimes for twenty years together, and in it a collection of spit, vomit, urine of dogs and men, beer, scraps of fish, and other filthinesses. Hence upon a change of weather a vapour is exhaled very pernicious to the human body. At inns people spat everywhere, and micturated on the floor or up the chimney. Some cleaned their boots at table. Wet clothes were put to dry on the stove, and the room reeked.[2] The French memoirs of the seventeenth and eighteenth centuries are full of disgusting details of the insanitary state of Paris and of the palace itself, where visitors relieved nature openly in the courtyards and even on the stairs. Stenches even pervaded the corridors of Saint-Cloud after the restoration in the days of Louis XVIII, and were extolled by an old faded Court beauty as

[1] Bernan, *History and Art of Warming and Ventilating*, London, 1845.
[2] *De civilitate puerili.*

recalling the good old times. Howard,[1] the prison reformer, regarded the poisonous effluvia of the horrible dungeons he visited as the cause of 'gaol' (typhus) fever which cleared off not only the prisoners, but not infrequently members of the bar and bench who tried them. He cites Bacon as saying: 'The most pernicious infection next to the plague is the smell of a jail.' In 1750 the Newgate prisoners infected the Court and forty died, including two judges and the Lord Mayor.

Not only prisons but hospitals were filthy and swarming with vermin. Howard found the inmates badly clothed, destitute of proper bedding, badly fed, and kept in close confinement. A surgeon named Day, following Howard's teaching, stamped out gaol fever at Maidstone prison by washing the walls and ceilings with boiling lime, treating the privies and drains with quicklime, changing linen and clothes, and washing the prisoners daily with warm suds, drying the clothes once a week upon an oast of sulphur, and giving nutritive food.[2]

The pioneer of sanitary reform of the Victorian era, Chadwick, concluded that the various forms of epidemic, endemic, and other disease are caused or aggravated or propagated chiefly amongst the labouring classes by atmospheric impurities, produced by decomposing animal and vegetable substances, by damp and filth, and close and crowded dwellings, and that the frequency and intensity of such disease are abated by means which diminish atmospheric impurity. Florence Nightingale maintained that fevers—typhus, typhoid, small-pox—were formed *de novo* and grew up in close crowded rooms or wards—evolved out of filth.

It is now recognized that infectious diseases are of microbic origin and spread not by poisonous exhalations but by (1) direct contact of an infected part of one man's body with the body of another, e. g. syphilis, gonorrhoea, infection of wounds by dirty fingers; (2) spray infection—the direct transmission by saliva spray of microbes from the respiratory membrane of one to that of another, e. g. common catarrhs, influenza, pulmonary tuberculosis, scarlet fever, diphtheria, measles, mumps, rheumatic fever; (3) dust from bodies or clothes of carriers of disease, e. g. the infection of food by the hands of the milk-man or cook who are carriers of typhoid fever, or dysentery. Out of 1,397 cases of typhoid in South-West Germany infection was traced to contaminated hands in at least 1,315. Fingers or clothes soiled with infected urine or faeces do the work. Walls of water-closets may be sprayed by the flush with infected material. *B. typhosus* and *B. dysenteriae* (Flexner) remain viable in a dried form for 3–4 weeks.[3] (4) Infection of drinking-water by human evacuations containing pathogenic microbes, e. g. typhoid fever, cholera. (5) Flies which transfer infecting microbes from evacuations of carriers of infectious disease to food, &c.; (6) biting insects which directly transfer infecting microbes from the blood of a carrier to another, e. g. plague spread by the rat flea, malaria and yellow fever by mosquitoes, typhus fever by lice.

It is also recognized that infecting microbes may be exalted in

[1] *State of Prisons of England and Wales*, London, 1792.

[2] Sweating, 'The Sanitation of Public Institutions,' *Howard Prize Essay*, London, 1884.

[3] B. Heyman, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1918, 86, 245.

virulence in some and attenuated in other conditions, possibly of external environment, certainly of the humours of the host; that virulence may be enhanced by injection of some chemically active material, or by mixed infection one microbe may enhance or diminish the immunity of the body to another; that immunity may be gained in some cases by vaccination with attenuated strains, e. g. small-pox vaccine, or the dead bodies of microbes, e. g. anti-typhoid vaccine, in other cases by inoculation with the humours of those immunized by vaccination, e. g. anti-tetanus serum, in still other cases by the injection of some specific chemically active substance, e. g. salvarsan. It has become clear that the suppression of plague, malaria, yellow fever, typhus, relapsing fever, depends on the suppression of biting insects—of the specific carriers of these diseases. The great fall in mortality from malaria and yellow fever in the tropics has been brought about by the cleaning up of the breeding-places of mosquitoes in the environment of human dwellings. Yellow fever has thus been almost abolished from America, and malaria from many places. Typhus fever is kept away by body cleanliness, typhoid fever and cholera by clean handling of food, pure water supply, and detection and discipline in cleanliness of carriers. Scarlet fever, measles, &c., can be kept down by free ventilation and the detection and isolation of carriers in the first stage of fever; the catarrhal infections by free ventilation and by vaccines; tuberculosis by open-air exercise and adequate rest and food and isolation of carriers.

It has been reserved for Teutonic culture again to bring upon Central Europe all the horrors of jails and hospitals detailed by the sanitary reformers a century ago. Filth and bad feeding have again made prevalent typhus, scurvy, rickets, tuberculosis, &c. Be it noted, however, that typhoid fever has been prevented in the face of all the foul conditions of trench warfare, the drinking of contaminated water, plague of flies, &c., by Almroth Wright's vaccine, that typhus was cleaned up in Serbia by the methods of stamping out body-lice introduced by English and American medical officers, by such methods as Day used in Maidstone Jail a century ago.

Be it noted, too, that catarrhal complaints, excluding the influenza epidemic, affected the army at the front, in spite of the conditions of trench-warfare, far less than the civilian population at home.

It was made abundantly clear that the well fed and clothed soldier has enormous resisting powers to exposure, putrescent filth, and stench, so long as he lives largely in the open air, is well exercised, kept lice-free, and vaccinated against small-pox and typhoid fever—a double vaccination which according to the present-day views of Almroth Wright increases the general immunity to infections.

In spite of all the exact knowledge that has accumulated as to the microbic origin of infective disease, and the ill-health that results from heat stagnation, it is still popularly taught and believed that much of the mischief resulting from the inspiration of re-breathed air is due to some subtle organic poison exhaled from the body, and the evidence for the existence of such must now be examined.

F. A. R. Russell[1] says: 'As to the frequent emission of a deadly particulate poison, no doubt whatever can exist. It is a dangerous

[1] *Smithsonian Misc. Collections*, 1896, **39**, 44.

and pernicious element in all aggregations, and, combined with carbon dioxide, produces, when in moderate quantity, depression, headache, sickness, and other ailments; when in large quantity, as in the Black Hole of Calcutta . . . rapid death in the majority . . . Much of the mortality of infant and adult life may be due to the rebreathing of poison excreted by breath and skin.'

It is commonly taught that it is necessary to keep the CO_2 in rooms below 0·1 per cent., so that the organic poisons may not collect to a harmful extent. The evil smell of crowded rooms is accepted as unequivocal evidence of the existence of such. The smell arises from sweat; from foul breath arising from food eaten, e. g. onions, garlic, from carious teeth, dyspepsia, and the decay of food particles on an unhealthy mucous membrane of the tongue; from the passage of wind from the bowel; from dirty clothes soiled with sweat, food, menstrual discharge, faeces, urine, &c. The clothes, too, absorb the smells of the stable, tobacco, &c., and give these out. The air of rooms may also be contaminated by smell of dirty floors and walls, of cooking, or that arising from latrines, by trade smells, &c. The smell of a crowded room is only sensed by, and excites disgust in, one who comes to it from the outside air. He who is inside is unaware of the smell. Flügge pointed out with justice that while we naturally avoid any smell that excites disgust, yet the offensive quality does not prove its poisonous nature. For the smell of the trade or food of one man may be loathsome to another. The sight of slaughter and the smell of dead meat and offal may be loathsome to the sensitive lady, but butchering is a healthy trade.

The stench of glue-works, fried-fish shops, soap and bone-manure works, middens, sewers, becomes as nothing to those engaged in or on such, and the lives of the workers are in no way shortened by the stench they inhale. The olfactory sense organ ceases to respond to the uniformity of impulse, or becomes fatigued. On descending into a sewer, after the first ten minutes the nose ceases to smell the stench; the air therein is usually much freer from bacteria than the air of schoolroom or tenement (Haldane). Sewer men are as healthy as other town labourers. It may be noted here that the splashing of sewage may spray bacteria for yards around, just as bacteria are spluttered from the mouth or sneezed from the nose (W. Horrocks).

With every change of barometric pressure there is a change of air in the ground and all spaces unless hermetically sealed. Smells thus come out of sewers when the barometer falls, and the fluctuation of barometric pressure carries dust and germs into shut up places.

The smell of fresh paint is usually considered unhealthy, but cats remained unaffected when kept in boxes the inside of which was freshly painted every day.[1] The lead poisoning of painters arises not from smell of paint but from inhalation of dust from scraping and rubbing down dry coats of paint.[2] The smell of alcohol or of cheese is horrible to a child, while the smell of putrid fish, which charms the native of the north Siberian coast, excites no less disgust in the epicure who welcomes the Stilton or Camembert cheese. Among the hardiest and healthiest of men are the North Sea fishermen, who sleep in the

[1] B. Moore, *Brit. M. J.*, Aug. 2, 1913.
[2] Legge and Goadby, *Lead Poisoning*, E. Arnold, 1912.

cabins of trawlers reeking with fish and oil, and for the sake of warmth (after hours of exposure on deck) shut the cabin up so that the lamp may go out from want of oxygen, i. e. the oxygen sinks below 17 per cent.; a percentage above this is necessary to support a flame. The stench of such a cabin puts a sensitive brain-worker off his appetite, and helps to make him sea-sick, but the robust health of the fishermen proves that this effect is nervous in origin and not due to some poison in the air absorbed into the blood. The labourer, or the lumber-jack, who is outside in the fresh air all day, also needs a warm room after he comes in at night. This is because of the great expenditure of energy by day. He is fatigued, and the high temperature then gives a feeling of rest and satisfaction. Apart from the greater risk of infection when influenza epidemics, &c., are flying round, it is not only natural but healthy for him or the fishermen to be as warm as possible by night. It is the sedentary indoor worker who needs to sleep with a window open.

The influence of the imagination is very great in nervous people. As an example I may cite the case of an institution where the lady clerks in a third-story room complained of being poisoned by fumes—turned sick and faint. I found the fumes escaped from a furnace room in the basement up the well of a lift, and from the top of this well passed to the fireplace of the room in which the clerks were. The fumes escaped when the furnace man raked out clinkers from the furnace. He must have breathed far more of the fumes, but made no complaint, and found he kept good health in his occupation. There is, too, the evidence cited in Part I, p. 149, that those who breathe fumes of sulphurous acid, &c., escape from influenza and cartarrhs. People of nervous temperament who suffer from chronic bronchitis and asthma appear to be especially sensitive to fumes of combustion, cold air, &c.

Studying the ventilation of sleeping cars T. R. Crowder found that in 'close' or 'stuffy' cars the temperature was always high. The most offensive smell was noticed in a day coach where 'the air was of such a degree of chemical purity as to indicate ideal ventilation by any standard that has ever been proposed'. The car was hot and had many filthy people in it. Perfect comfort has been found associated with the highest chemical impurity in other cars.

The right way of dealing with an offensive smell is to remove its cause. As Pettenkofer said: 'If there is a dungheap in a room it must be removed. It is no good trying to blow away the smell.' In school, hospital, or house, an open window in a foul latrine may cause the impulsion of the smell into the house. Houses, clothes, and bodies of people must be made clean.

Deodorization by ozone is one of the simplest methods of dealing with offensive trade smells. Ozonized air is used to take away or diminish the unpleasant smell of the tube railways. It must be added to the air in such dilution as to be just perceptible to the smell, in stronger concentrations, e. g. 1 in 10 million parts, it acts as an irritant poison.

Ozone in bearable concentration is of little value as a bactericide, but by slightly irritating the respiratory mucous membrane and increasing the outflow of secretion it may help to keep off catarrhal infections. An ozone concentration of 3 mg. per cub. m. of air (1·4 in

a million by volume) was sufficient to kill in 3 to 4 hours more than 95 per cent. of the bacteria present on the surface of a culture medium. But as ozone penetrates with difficulty, colonies were hardly affected by this relatively high concentration. Low temperature favoured the action. Spores and mycelia of moulds were killed on the surface, but not the mycelium below the surface. Ozonized air by acting on the surface may help somewhat to improve the keeping of flesh in cold storage.[1] It takes many hours' exposure to 50 parts per million to kill moth in flour.

The so-called 'active oxygen' of fresh country air is not ozone but nitrogen peroxide, probably formed by ultra-violet rays of sunlight causing the direct union of nitrogen and oxygen.

An analysis of the so-called 'ozonmetric' records of many observatories over the last fifty years show significant relationships to the seasonal energy of the sun and to strong winds blowing from southern latitudes. From spring to autumn the ozonmetric chart follows approximately the daily amount of solar radiation, calculated from its recorded intensity and daily duration. The sudden rise in April coincides with the great burst in vernal activity on sea and land, towards which there is evidence that light is more important than rise of temperature of sea or air as the initial stimulus (B. Moore).

The widespread belief in the presence of organic poisons in the expired air is mainly based on statements of Brown Sequard and D'Arsonval.[2]

These statements have done very great mischief to the cause of hygiene, for they have led ventilating engineers to seek after chemical purity and neglect securing adequate cooling and evaporative power of the air. Their authors claimed that the water condensed from the breath and injected into animals acted as a poison. Some of their results, and those of one or two others who have brought forward confirmatory experiments, may be attributed to bacterial infection, some to the toxic effect which is known to follow injection of water containing bacteria,[3] and some to the very large doses of water which were injected at room temperature. For example, doses were injected into rabbits equivalent to 3 litres in a man, enough to make any animal ill.

A large number of investigators have repeated the experiments with condensation water, using proper precautions and with negative results. Billings, Weir Mitchell, and Bergey [4] made a very thorough investigation of this matter. They found that in ordinary quiet breathing no bacteria, epithelial scales, and particles of tissue are exhaled.

The minute quantity of ammonia or other oxidizable matter in the condensed water of the human breath appears to be due to the decomposition of organic matter in the mouth; it is greater 4 hours than half an hour after eating, and far less if the mouth is cleansed.

In the condensation water from a hospital ward the traces of

[1] L Hill and M. Flack, *Proc. Roy. Soc.*, 1911, **B. 84**, 404; R. Heise, *Arb. a. d. k. Gesundheitsamte*, 1917, **50**, 418; J. Edkins and N. Tweedy, *Roy. Soc., Reports of Grain Pests Committee*, No. 4, 1919.

[2] *Compt. rend. Soc. de biol.*, 1887, **4**, 814; 1888, **5**, 33, 54, 94, 108, 121.

[3] McIntosh and Fildes, *Lancet*, March 9, 1912.

[4] *Smithsonian Contributions to Knowledge*, 1895, No. 989, also 1903, 29.

ammonia are connected with the presence of dust, bacteria, &c., which can be filtered off. The bacteria are the only really dangerous elements in the air. The condensed moisture of the human breath collected in sterilised apparatus, and warmed to body temperature, had no toxic effect when injected into animals.

Weichardt in 1911 claimed that he had found evidence of the toxic properties [1] of moisture condensed from the breath taken up by blotting-paper in a room crowded with sleepers, but Inaha [2] conclusively showed that the same symptoms of poisoning were caused by equally large doses of distilled water. Weichardt injected into mice as much water as would be equivalent to injecting .5 litres into a man, an absurd experiment!

Rosenau and Amoss made experiments on breathing for some hours through a plug of glass wool into a flask wherein the water of the breath was condensed by cooling with ice. The fluid was injected into guinea-pigs, and a month later a trace of human serum was injected into the same animals. Anaphylactic shock resulted, showing that the condensation water contained human protein which sensitized the animals. The glass wool in such an experiment must become wet through, and drops of saliva be carried through into the flask. The guinea-pigs were then sensitized by traces of salivary protein, and such experiments prove nothing as to the presence of an organic poison in the breath.

M. Flack, J. McIntosh, and the writer [3] kept white rats and guinea-pigs together at the bottom of deep boxes with the lid so closed that the animals lived in an atmosphere vitiated with CO_2 to the extent of 0·5–1·5 per cent., and breathed each others breath, just as a crowd of people in an ill-ventilated room. The boxes were cleaned daily, and the animals well fed. After so living for many weeks rat's serum was injected into the veins of the guinea-pigs. In no case was there any evidence of anaphylaxis. A month later a second dose of rat's serum killed the guinea-pigs (now sensitized by the first dose) by anaphylactic shock.

The evidence seems to show that certain neurotic and asthmatic people are sensitive to the presence of a dog, or cat, and can detect the animal when concealed in the room. In the case of an asthmatic sensitive to horses, the effect of touching first the skin of a horse and then the conjunctiva of his eye, produced a violent catarrh lasting an hour. This man was upset by eating a sausage in which horse meat was introduced. These cases are allied to those of specific sensitivity to some food protein. Some people vomit on eating egg, crab, mussels, &c., some are attacked with nettle rash.

Some asthmatics cannot bear the heavy scent of flowers, e.g. white narcissi or white may. The olfactory sense and respiratory membrane are both sensitive to extraordinary dilutions of certain substances. Thus in the case of the irritating fumes of certain arsenic compounds used in warfare as little as 1 part in 500 million of air is irritating to the nose. The particles of the fume, some 1 μ in size, may be detected by the ultra-microscope. In the case of thioacetone,

[1] *Arch. f. Hyg.*, 1911, **74**, 185.
[2] *Zeitschr. f. Hyg. u. Infektionskrankh.*, 1911, **68**, 1.
[3] *Smithsonian Miscellaneous Collections*, 1913, **60**, 1.

said to be the most offensive of odours, the smallest trace in a million cubic metres of air can be detected. 1^{-15} of a gramme of musk can be detected by smell according to Bertholet.

C. E. A. Winslow and D. Greenberg [1] have exposed young guinea-pigs to the odour of fresh human or dog faeces, drawing the air over such into the boxes where the animals were confined so as to produce a strong faecal odour therein. The results seemed to indicate that the breathing of putrid gases causes a reduction in the rate of growth during the first week of exposure; but that this effect was a transitory one, the exposed animals gradually becoming accustomed to the odour and attaining after two weeks a normal weight. It is to be expected that guinea-pigs would be frightened by the smell of excreta of dog or man, and might be put off their feed thereby to start with.

Brown Sequard and D'Arsonval claimed that animals died from poisoning when confined in chambers through which air was drawn from a series of other chambers containing animals, so that exhaled breath was breathed.

It has been proved by exact repetition of these experiments by several competent researchers that, so long as the oxygen is not allowed to sink to limits incompatible with life or the CO_2 to rise to concentrations which are poisonous, animals remain in good health.

I. Kianizin [2] claims that life is impossible in the absence of saprophytic bacteria, since in their absence the formation of oxidizing ferments is so much reduced that auto-intoxication takes place. Sterilization of the air, he finds, reduces the oxidation processes in rabbits placed in a D'Arsonval apparatus, and he thinks similar results may be produced by the roasting of air in central heating. As his control animals do not appear to have been confined in a D'Arsonval apparatus, low cooling power and not sterilization of the air may well have been the cause of the reduced oxidation (tested by the urinary constituents).

The evidence, then, in favour of the presence of organic poison in exhaled air or air of crowded, ill-ventilated rooms is negative. The physical conditions of the atmosphere and infecting microbes are the sole causes of ill effect of such on health. The changes in oxygen and CO_2 content which occur in crowded rooms are not such as to produce ill-health. The evidence that this is so will now be considered.

Analyses show that the oxygen in the worst ventilated school-room or public meeting-place is never lessened by more than one per cent., and very rarely as much. Natural ventilation is kept up by the increase of temperature and moisture within, which lighten the air, and by the wind outside. This taking place through every chink and cranny, chimney opening, porous brick wall, and plaster ceiling, prevents a greater alteration.

Changes of barometric pressure outside and of the density of the air in hot, moist rooms have considerably greater effect on the partial pressure of oxygen.

The barometer varies at sea-level between 28 and 31 inches (710–785 mm.).

Taking the highest figure and supposing the air dry and at 0° C. the pressure is 785 mm. If the temperature of the air is

[1] *Proc. Soc. Exp. Biol.*, 1918, 123. [2] *J. Physiol.*, 1919, **52**, 416.

raised to 30° the air will expand and the mass which occupied a volume V_0 at 0° C. will occupy a volume $V_0 \times \frac{303}{273}$. The volume V_0 could have been caused to expand to this amount if the temperature had been kept at 0° C. and the pressure decreased from p_0 to $p_0 \times \frac{273}{303}$. Taking the lowest value given above, 710 mm., when the temperature has been raised to 30° C. the air will be of the same density as if the pressure was $710 \times \frac{273}{303} = 640$, i.e. equivalent to $(785 - 640) = 145$ mm. less pressure than under the high barometer conditions or 18·5 per cent. reduction of pressure.

Suppose the total pressure to remain the same, but 'f' mm. be due to water vapour, in the case considered above the density of the dry air will be equivalent to a pressure $(640 - f)$. The value of 'f' for air with dewpoint say 25° C. is 24 mm.; in such case the pressure will be $640 - 24 = 616$ mm., or reduction on high barometer and dry air conditions at 0° C. of 169 mm. or 21·5 per cent.

People would breathe, then, in hot, moist rooms on a day of low barometer air with a partial pressure of oxygen 20 per cent. less than in the frosty air outside on days of high barometric pressure. With each pint of air they breathed they would inhale a fifth less oxygen by weight.

Such a diminution is easily made good by a slight increase in the volume breathed, but it must be borne in mind that any such increase of pulmonary ventilation lowers the partial pressure of carbonic acid both in the lung and in the blood, and this in its turn has to be compensated for.

In crowded, hot rooms where the heat-regulating mechanism is strained, the blood pooled in the cutaneous capillaries, the heart rapid, and the breathing rapid and shallow, the fall in oxygen partial pressure may contribute to the oppression of feelings, particularly in those who suffer from heart defects or vasomotor instability. Similarly a cool atmosphere favours cases of pneumonia suffering from oxygen want. Such do badly at high altitudes and are benefited by oxygen inhalations.

When the breathing is shallow the blood may not be properly oxygenated, as much of the inspired air does not reach the alveoli.

Mountain sickness is more frequently experienced in thundery weather by those who travel by train to the Andes. The best day for arrival is clear, dry, cold weather. Those who take alcohol on the journey up suffer worse than those who do not.[1] The close weather or alcohol causes flushing of the cutaneous vessels and so increases the want of oxygen.

At sea level the partial pressure of oxygen in the air is 150 mm. Hg; in the pulmonary alveolar air about 100 mm. Hg. Taking haemoglobin as saturated when shaken with air it is 99·8 per cent. saturated at 90 mm. oxygen partial pressure, 99·5 per cent. at 80 mm., 98·8 per cent. at 70 mm., 97·6 per cent. at 60 mm., 95·4 per cent. at 50 mm., 90·4 per cent. at 40 mm.

[1] T. E. Ravenhill, *J. Trop. M.* [*etc.*], Oct. 15, 1913.

In noted health resorts of the Swiss mountains the barometer stands at such a height that the partial pressure of oxygen is less than in the most ill-ventilated room at sea-level. On the high plateau of the Andes there are great cities, mines are worked, and railways built. Potosi, with 100,000 inhabitants, is at 4,165 metres, and there girls dance half the night and toreadors display their skill in the ring. The Indians of the Andes are a hardy race with broad, long, and capacious chests. On the slopes of the Himalayas Thibetans, no less remarkable for vigour and activity, take their flocks to altitudes of 18,000 feet.

TABLE I.—*Variation of Atmospheric Pressure and Temperature with Altitude*, J. Harker.

Ground level 30″ = 762 mm. Hg and 48° F. or 9° C.

Height in feet	Pressure				Temperature	
	lbs. per sq. in.	Inches of mercury	Mm. of mercury	Atm.	°Fahr.	°Cent.
0	14·72	30	762	1·0	48	9
2000	13·65	27·8	706	0·929	44	7
4000	12·70	25·9	657	0·864	40	4
5000	12·20	24·9	632	0·832	37	2
6000	11·75	23·9	608	0·799	34	1
8000	10·85	22·1	561	0·738	29	− 2
10000	10·05	20·5	520	0·684	23	− 5
12000	9·30	18·9	481	0·633	17	− 8
14000	8·55	17·4	442	0·582	9	−13
15000	8·20	16·7	425	0·560	5	−15
16000	7·90	16·1	408	0·537	2	−17
18000	7·25	14·8	375	0·493	− 6	−21
20000	6·65	13·5	344	0·452	−14	−26
22000	6·10	12·4	315	0·415	−22	−30
24000	5·60	11·4	289	0·381	−30	−34
25000	5·35	10·9	276	0·364	−34	−36
26000	5·10	10·4	264	0·347	−37	−38
28000	4·65	9·5	240	0·316	−45	−43
30000	4·25	8·6	220	0·289	−52	−47

Temperature at high altitudes varies with the ground level temperature in summer and winter.

TABLE II.—*Relation of the Volume of a Gas measured at Various Heights to its volume at Ground Level*, J. Harker.

Height in feet	Volume occupied at ground level by 1 litre taken at height specified	Volume occupied at height specified by 1 litre taken at ground level
0	1	1
5000	0·853	1·17
10000	0·720	1·39
15000	0·611	1·64
20000	0·517	1·93
25000	0·432	2·31
30000	0·361	2·77

Note. In computing this table the pressures and temperatures as given in Table I have been employed. If the actual ground temperature varied from the assumed value (9° C.) by 10° C. the figures in columns 2 and 3 would not be changed by more than 1 per cent., and

the alteration in these ratios due to ordinary departures of the atmospheric pressure from the value assumed (30″ or 762 mm.) would be of an even smaller order of magnitude.

The new-comer who is carried by the railway in a few hours to the top of Pike's Peak or the Andes may suffer severely from mountain sickness, especially on exertion, and the cause of this is want of oxygen. The common symptoms are sleeplessness, nocturnal attacks of breathlessness, abnormal rapidity of pulse after exercise, vomiting, and fainting. Cases of heart disease may collapse and die in the train on being carried rapidly to these high altitudes. Owing to the increased pulmonary ventilation, excited by want of oxygen, CO_2 is washed out

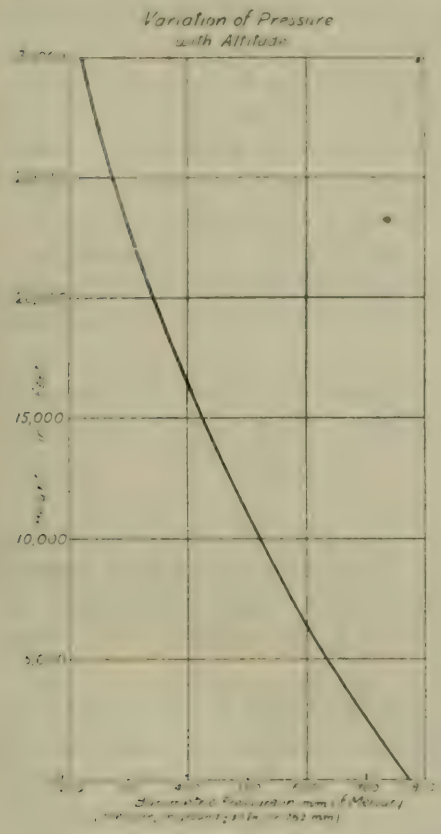


Fig. 16.

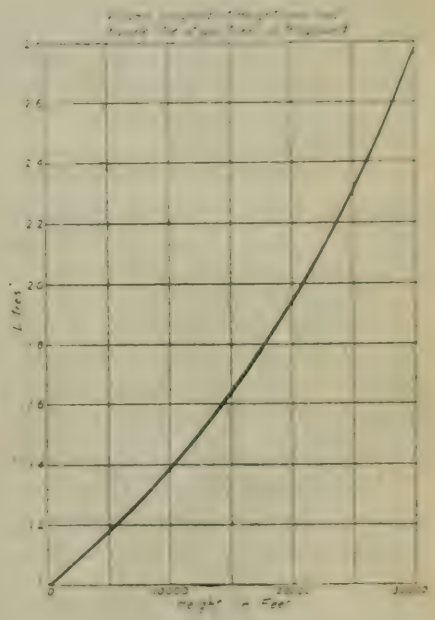


Fig. 17.

of the body, and a state of alkalosis of the tissues produced in the unacclimatized. Not only breathing of oxygen, therefore, but of 1–2 per cent. carbonic acid relieves the symptoms.

Acclimatization is brought about by the hydrogen-ion concentration of the blood being suitably adjusted by renal action, while the carbonic acid partial pressure is reduced in the alveolar air of the lungs, that of oxygen being raised proportionately by increased pulmonary ventilation; the kidney making suitable adjustment by controlling excretion alkalosis is prevented, and the normal neutral reaction of the tissues secured. The number of red corpuscles and total quantity of haemoglobin are increased, and thus the carrying power of the blood for oxygen made greater. J. S. Haldane[1] maintains that oxygen may be

[1] *Organism and Environment as Illustrated by the Physiology of Breathing*, New Haven, 1917.

actively secreted into the blood by the pulmonary epithelium—the swim bladder of certain fishes can secrete oxygen. There is, however, no evidence of a glandular structure of the epithelium of the lung such as there is in the swim bladder ; and recent observations are against its secretory action, see page 214.

As each cubic metre of air holds the same amount of water vapour at high altitudes as at low when saturated, far more evaporation takes place from the respiratory membrane at high altitudes, the air being relatively drier and a greater volume of cool air being breathed there. To counteract the greater cooling and drying more blood, lymph, and secretion will flow through the respiratory membrane at high altitudes, and in this lies part of the beneficial effect of Alpine health resorts.

In the case of people at sea-level, when the partial pressure of oxygen in the air is made to fall quickly to 14–12 per cent. of an atmosphere, hyperpnoea begins to be noticeable ; at 10 per cent. it becomes distinct, capacity for mental action and acuteness of sensation are lowered, the reaction time lengthened, cyanosis becomes evident, the pulse-rate increases, and there is a rise in systolic and fall in diastolic pressure, with increased throbbing of the heart and arteries. Anoxaemia causes increased secretion of adrenalin and formation of sugar by the liver, these effects being brought about by stimulation of the splanchnic nerves.[1] At 5 per cent. cyanosis is very marked and consciousness lost.

On breathing deoxygenated air consciousness is lost in some 45 seconds, and without warning. Convulsions follow with a temporary rise of blood pressure, followed quickly by a fall, cessation of respiration and of the heart beat.

Animals may live in an atmosphere, by gradual acclimatization, in which the proportion of oxygen has become so low and that of carbonic acid so high that a similar animal, brought from fresh air into it, dies almost at once.[2] The degree of this immunity to oxygen want varies with individuals.

Haldane found that men became acclimatized to 9·5 per cent. oxygen by 56 hours spent in the sealed atmosphere of a submarine. Those coming in fresh from the outside air suffered from oxygen want. Diffusion is far freer in rarified air, and thus a partial pressure of oxygen of 9·5 per cent. at an atmosphere is worse at sea-level than at high altitudes. The nitrogen molecules in rarified air do not impede the movement of the oxygen molecules as they do in dense air. In compressed-air chambers used for tunnelling under water, and supplied to divers, the oxygen partial pressure is raised. At +2 atmospheres the oxygen is equivalent to 63 per cent. at sea-level. Work is made easier and fatigue kept off by the higher pressure of oxygen.

The athlete suffers from want of oxygen in the performance of very strenuous efforts whenever he uses up oxygen in his muscles more rapidly than it can be supplied by respiration and circulation. A man doing hard exercise, pushing a heavy motor-cycle up a steep hill, may use up 3,000 c.c. of oxygen per minute, and when swimming 3,500 c.c. against 300 c.c. when resting. The athlete in these conditions, particularly if out of training, is like the mountain-sick

[1] C. H. Kellaway, *Proc. Physiol. Soc.*, p. xliii, *J. Physiol.*, 1919, 52.

person or air pilot at lofty altitude. He is benefited greatly by oxygen inhalation. So, too, are cases of pneumonia, or of irritant pulmonary gas poisoning, suffering from oedema of the lungs and obstruction to oxygen supply, or cases cyanosed from defective action of the heart, or cases poisoned by carbon monoxide or nitrites, which reduce the oxygen-carrying power of haemoglobin.

In conditions of shock too, or faintness from exposure to excessive heat, where the blood is pooled in the capillaries, oxygen inhalation is of value.

The regularity, strength, and fullness of the pulse are notably restored by oxygen inhalation, and the signs of cyanosis removed.

Breathing oxygen not only ensures the better saturation of the haemoglobin passing through the lungs (in quick shallow breathing parts of the lungs may not be ventilated), but adds 2 per cent. more oxygen which is simply dissolved in the plasma in accordance with the partial pressure.

Nitrous oxide and oxygen are recognized as affording the best means of anaesthetizing cases of wound shock.

Oxygen breathed by a runner just before the pistol fires makes him run faster and easier, and breathed again just after the race stops feelings of stiffness and fatigue. It also relieves the dyspnoea and restores the vigour of a spent boxer given between the rounds (Hill and Flack). It reduces the frequency of the heart beat four or five beats per minute in the normal resting individual.

Cases of pneumonia and failing heart are sent down from high altitudes, such as the Rand and Mexico City, to the plains, for it is recognized this gives the best chance of recovery. The continued treatment of such cases, and cases of surgical shock, in chambers with the atmosphere enriched up to 30 per cent. of oxygen now requires trial.

Carbon monoxide poisoning is due to oxygen want, the former gas displacing the latter from its combination with oxygen. The haemoglobin of a man at rest may become one-third saturated with carbon monoxide without his realizing anything is wrong, but if he makes any considerable exertion he collapses from oxygen want, the demand for oxygen being then so much greater.

Carbon monoxide has an affinity for haemoglobin some 200 times greater than oxygen, and thus it is dangerous to breathe for long an atmosphere containing 0·1 per cent., for this will produce half-saturation. J. S. Haldane states that breathing 0·04 per cent. will *finally* produce about one-third saturation, 0·08 per cent. one-half, 0·16 two-thirds. It requires 600 c.c. of CO to combine with the blood to produce prostration. Breathing 0·3 per cent. for 15 minutes (at the normal resting rate of pulmonary ventilation) will effect this. A man who has breathed 0·2 per cent. for four or five hours, or 0·4 per cent. for one hour, will generally die. In air containing 2–5 per cent., as after an explosion of coal dust in a mine, death follows almost as quickly as in drowning.

It was shown by Leblanc in 1842 that an animal can breathe with impunity an atmosphere containing as much as 30 per cent. CO_2 for three-quarters of an hour provided that the percentage of oxygen is 70.

Billings, Weir Mitchell, and Bergey [1] found that at a temperature

[1] *Smithsonian Contributions to Knowledge*, 1895, 39.

of 50° C. air rich in oxygen supports the life of animals confined in a chamber wherein exhaled CO_2 accumulates much longer than ordinary air. This, they say, was not the case at 0° C. The accumulated CO_2 perhaps counteracts the polypnoea produced by the hot atmosphere and keeps the breathing deep.

With the lungs full of oxygen it is possible to hold the breath until the partial pressure of CO_2 in the lungs reaches 10 per cent., or even more, while with air in the lungs the breaking-point is at 6–7 per cent. (Hill and Flack). It seems probable that the oxygen, by preventing formation of lactic acid in the respiratory centre and heart, staves off the breaking-point.

In 1860 Pettenkofer laid down the rule which has been taught by sanitarians for 60 years, that the percentage of CO_2 is a valuable indicator of the purity of the atmosphere.

It is generally supposed that the excess of carbonic acid in the air of ill-ventilated and crowded rooms acts as a poison. No supposition could be further from the truth. The partial pressure of CO_2 in pure air of the country may be taken as 0·03 per cent. of an atmosphere, or 3 parts in 10,000. Out of doors in London it averages 0·04. In the worst ventilated theatres, courts of law, schools, &c., it does not run above 0·5 per cent., or at the outside in most exceptional cases 1 per cent.

It is impossible that any such excess should enter into our bodies when we breathe such air, because the air in the pulmonary alveoli is kept constant at about 5–6 per cent. of an atmosphere by the action of the respiratory centre.

The concentration of CO_2 in the blood rules the respiratory centre in the brain, and to such purpose as to keep constant the concentration in the blood and in the lungs (Haldane and Priestley). The only result of breathing 0·5 per cent. CO_2 is a slight and unnoticeable increase in the ventilation of the lungs, just as happens when we move about and produce a little more CO_2 in our muscles.

When the CO_2 percentage rises over 3 per cent. hyperpnoea begins to be troublesome, and there may be slight headache. On breathing 5–6 per cent. the hyperpnoea is exhausting,[1] the face flushes, and there is sweating and headache. Breathing 10 per cent. of CO_2 cannot be endured for more than a minute or so; there results headache, sweating, dimness of vision, and tremors.

On inhaling 5 per cent. it is possible to exhale 6 per cent. and keep up a respiratory exchange by great hyperpnoea; beyond this point symptoms of poisoning from CO_2 rapidly increase as the output from the body is checked.

At each breath we breathe into our lungs the air in the nose and large air tubes (dead space air) contaminated with expired air. To breathe some of the air exhaled by others is the common lot of men and animals who crowd together in close shelters to husband their heat in cold weather. White rats will stay in their sleeping chamber when this is so ill-ventilated that the CO_2 percentage rises to 5, but when the air is made hot and moist they come outside.

Cadman and Whalley reported a place in a coal mine where a quantity of black-damp was given off and where a light would not

[1] J. F. F. Hermans, *Arch. f. Hyg.*, 1883, **1**, 1.

burn 1 ft. 6 in. from the floor, or in the waste, but the men had no fault to find with the air. The foreman said it was better than usual.

Analyses of samples taken (1) at the floor, (2) at the coal face, showed:

		per cent.		per cent.
(1)	CO_2	4·56	O_2	13·13
(2)	,,	1·21	,,	18·97

Men in submarines are willing to remain submerged until the CO_2 rises to 3 per cent.

Men in breweries work for years in places where the CO_2 may be 1–2 per cent. and retain excellent health.

Two per cent. increases the pulmonary ventilation about 50 per cent., 3 per cent. about 100 per cent., 5 per cent. about 300 per cent. Such increases lessen the working power in so far as the limit of hyperpnoea is sooner reached.

Divers constantly do heavy work in air with more than 1 per cent. CO_2. It is important to bear in mind in the case of divers and compressed air workers that it is the partial pressure which matters, not the percentage. One per cent. at a pressure of three atmospheres equals 3 per cent. at one atmosphere. At two atmospheres (33 feet) the ventilation of the diver's helmet must be double that required at one atmosphere, at three atmospheres treble, and so on (Haldane).

F. C. Benedict and R. D. Milner[1] give records of a subject who lived in their respiration chamber for 24 hours in an average of CO_2 over 220 parts per 10,000—over 70 times normal. He was comfortable, and the respiratory exchange and the heat evolution were practically the same as on the preceding day when the CO_2 was kept down to 2–10 parts per 10,000. 'It is certain that no differences in the respiratory exchange and heat output are to be noted which at all correspond to the enormous differences in carbon dioxide content.'

The heat absorber in the chamber was cooled below the dewpoint of the air so that the moisture condensed and the humidity was held within reasonable bounds. This condensation water injected subcutaneously into white rats was proved to be non-toxic. The chamber was kept at 20° C. (68° F.). The air entered the chamber very dry. The humidity averaged 67 per cent. It was approximately 75 per cent. during the work periods. The chamber was 2·15 m. long, 1·92 m. high, 1·22 m. wide, with a cubical content of approximately 4,800 litres. With no ventilation and proper cooling appliances no discomfort was felt when the subject remained inside several hours.

With 75 litres maximum ventilation per minute the subject remained inside 12 days, and varied his CO_2 output from 800 grm. on the days of rest to 1,400 grm. on the days of work. The velocity of air movement is only about 2 metres an hour, the air then is practically still (the dry kata reading comes out as 4·4, the wet as 12·9), with a humidity and temperature kept constant.

In the Monte Rosa hut (4,560 m.), owing to bad weather, there were confined for three days 45 persons in a space 9·2 m. × 3 m., giving each person 1·29 c.m. The stove acted as a ventilator and windows were opened from time to time to renew the air. Transient headache was the only ill-effect.[2]

[1] *U.S. Dept. Agriculture*, Bulletin 175, June 15, 1907, p. 260.
[2] A. Mosso, *Life of Man on the High Alps*, 1898, 62.

Benedict records (p. 255) a submarine boat with a crew of six men and a free air capacity of 2,250 cubic feet which was submerged for 15 hours. No fresh air was supplied or foul air removed, but the temperature of the water outside kept the temperature and vapour pressure low, water being condensed on the walls. Body heat in the still air was lost by radiation to the cold walls and by evaporation, the loss by convection being small. At the expiration of the test the interior of the boat was somewhat 'close,' like a 'stuffy' room, but there were

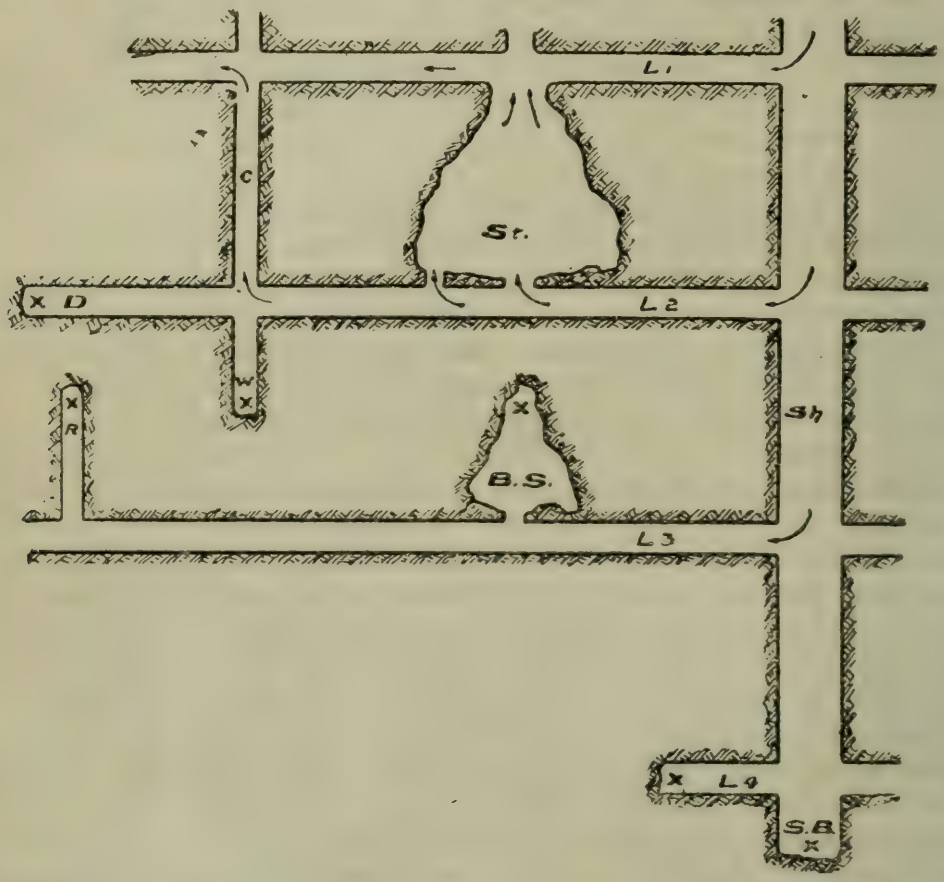


Fig. 18. Localities in a mine where gassing accidents are likely to occur (Irvine).

Sh = shaft for haulage of rock and men and upcast or downcast in ventilation.
L 1, 2, 3 = levels or 'drives', tunnels driven off from shaft, serving as tramways and main airways.
SB = bottom of shaft.
C = air connexion between two levels.
St = this has been opened out, forming a 'stope'.
D = blind end of a 'drive'.
W = tunnel being sunk from level to level but no through connexion established, called a 'winze'.
R = similar tunnel being raised upwards, a 'raise'.
B-S = a 'raise' worked out and forming a 'back-stope'.

absolutely no observable bad effects on the crew ; the men could endure concentrations of CO_2 up to $3\frac{1}{2}$ per cent. without great difficulty. The ventilation rate may be computed at 12 litres per minute per man. Divers constantly work for many hours in shallow water with a ventilation rate of about 60–70 litres a minute, and kept cool by the water suffer no discomfort from poor ventilation.

The ordinary standard for ventilation is 1,000 cubic feet of air space per person, and this to be changed every hour, that is 460

litres per minute; the diver habitually works with 60–70; the submarine crew had 12. These figures show strikingly how the problem of ventilation in ordinary rooms is one of attaining the right cooling power, not chemical purity of the air.

The important deficiency or impurities found in air of mines[1] are:

1. Deficiency of oxygen.
2. Excess of carbon dioxide.
3. Methane, 'fire-damp'.
4. Carbon monoxide.
5. Nitrous fumes.
6. Sulphuretted hydrogen and sulphurous acid.

Asphyxiation due to 'black-damp' or 'fire-damp' is due to want of oxygen, since methane is not poisonous, and carbon dioxide is seldom present in concentration enough to be a danger in itself. Black damp is the result of oxidation processes occurring in coal, timber, iron pyrites, &c., and may collect in any underground working where ventilation is deficient. Thus in wells deoxygenated air may collect, especially when the barometer falls and air comes out of the soil into the well. In explosions of fire-damp and coal dust in mine fires, 'after-damp' causes death, carbon monoxide being the source of danger. Spontaneous heating of coal is also a source of this gas. Where air compressors are used in mines over-heating of oil has occasionally produced carbon monoxide. Imperfect combustion of any kind in a closed space, e.g. charcoal stoves, and escape of ordinary commercial gas into a closed space, occasion poisoning from carbon monoxide. Sulphuretted hydrogen and sulphurous acid may cause irritation of the eyes and respiratory passage, but are scarcely ever present in dangerous quantities.

Fumes of explosives contain carbon monoxide and nitrous fumes, and are important causes of accidents. Whenever explosives are fired in closed spaces, imperfect detonation or accidental firing increase the amount of these dangerous gases greatly, which tend to collect in stagnant blind ends. Thus fumes produced by blasting in the end of a drive may drift into the nearest winze. There is danger in entering such blind ends unless first ventilated by compressed air hoses.

Gassing by explosives occurred in battleships, dug-outs, mines, and in steel 'pill boxes' at the front during the war. Deoxygenated air was also a trouble in the sinking of mines, and oxygen breathing apparatus was extensively used.

Perfect explosion of 1 lb. of blasting gelatine yields in cubic feet of gas (Irvine):

	CO_2	N_2	CO	NO
Burned in excess of air	5·7	2·3	0·7	1·2
Burned in vacuo	—	—	6·0	5·0

Death results if a man, rendered unconscious by carbon monoxide, be not soon extricated and removed to fresh air. Those who then survive the first hour or two usually recover. Sudden exposure to cold air may intensify the symptoms no doubt by increasing oxidation

[1] For references cf. L. G. Irvine, *Med. J. South Africa*, Sept. 1915; J. S. Haldane, *Investigation of Mine Air*; J. Glaister and D. D. Logan, *Gas-poisoning in Mining and Other Industries*.

processes for heat production. Warmth is then necessary. The bodies of those poisoned have pink cheeks and lips, the cherry-red colour of carboxy-haemoglobin.

Guinea-pigs are not incommoded by a 25 per cent. saturation of their blood by carbon monoxide, and can gradually be brought to tolerate 45 per cent. saturation, an initial extensive degeneration of red corpuscles, accompanied by a short period of ill-health and loss of weight being rapidly compensated for by a great increase in the number of red corpuscles. Polycythaemia similarly results in men who suffer from oxygen want due to altitude, chronic coal-gas poisoning, heart disease, &c. Excitability, irritability, loss of memory 'grip' and initiative, depression, breathlessness on exertion, palpitation and vasomotor instability, in some cases neuritis, and even paralysis from degeneration of the central nervous system, have resulted from severe or chronic carbon monoxide poisoning. Similar have been the results of severe pulmonary irritant gas poisoning in the late war, the pulmonary oedema so set up leading to oxygen want for many hours. Chronic carbon monoxide poisoning may occur in those who breathe in close places air contaminated by slow-combustion stoves and gas engines, or where gas escapes or is used as a fuel in stoves without flues.[1]

Bacterial toxins may have a similar effect, 'locking' the respiratory function of the tissue. The writer, when suffering from a headache due to some toxic absorption from the bowel, was unable to successfully mark with a pencil small circles irregularly placed on a tape which was kept moving behind a slit. After breathing oxygen he was able to mark the circles with surprisingly greater success.

Small haemorrhages, thromboses, softings, degenerations, chiefly in the nervous and cardio-vascular system, are found in cases that die from carbon monoxide poisoning.

If an animal is placed in two atmospheres of oxygen it can be kept alive even though its haemoglobin is saturated with carbon monoxide. Enough oxygen is then dissolved in the plasma.

Oxygen breathing is then the proper means of resuscitation, and oxygen breathing apparatus is required to investigate mines filled with after-damp. Canaries and mice are used to test the air; they may be carried into the mine in a box supplied with oxygen.

Nitrous acid fumes cause a sense of irritation at the time of inhalation, smarting of the eyes, coughing, &c. These symptoms are not severe and commonly pass off (so that the worker feels quite well), to be followed in four to eight hours by acute oedema of the lungs and very often death. Phosgene used as a poison-gas in the war produced the same sort of insidious oedema.

There are about 10,500 whites and 123,500 natives employed on the Rand underground, and 80,000 lb. of explosives used daily in blasting, and over 30 lives a year lost from gassing, not counting the many cases of illness. The majority of them are due to carelessness in using the means of local ventilation (Irvine).

[1] 6 per cent. of COHb was found in the blood of one habitual smoker by Hartridge, *Proc. Physiol. Soc.*, Jan. 31, 1920, *J. Physiol.* 53.

CHAPTER II

The Smoke Nuisance and the Economic Use of Coal.

In the fourteenth century one John Smith was hanged for creating a smoke nuisance; in the twentieth century people are sometimes punished for river pollution, and for adulteration of food, but for polluting the air they are merely punished with a caution.

Professor W. A. Bone regards the smoke nuisance as a preventable source of damage to buildings, furniture, fabrics, human vitality, and plant life, which, if expressed in money values, would probably amount annually in Great Britain to a loss of 50 millions sterling or more; in addition, there is a prodigious loss of valuable by-products, which under scientific organization and control might be saved.

The material damage done by smoke to property in Manchester and Salford has been estimated at one million annually, or about £1 per head of population. A similar estimate for London is four million pounds.[1] Much of the damage is incalculable. Cohen and Ruston[2] found that the hours of bright sunshine were 17 per cent. fewer in the centre of Leeds than at the outskirts, and that the intensity of diffuse daylight was 40 per cent. less.

Thirty years ago the artisans in Hulme (Manchester) were estimated to receive 50 per cent. less actinic rays of sunlight than the people in Didsbury, four miles away.

The increase in London of gas heating and cooking appliances in the ten years 1902–11 was 1,300,000, an increase which may probably be correlated with the lessened number of fogs.

	Average hours of sunshine	Average no. of days of fog
1902-6	1257	27½
1907-11	1341	17

Comparing the 20-year averages of the monthly duration of bright sunshine for stations in London with corresponding values for stations in more rural situations, the following percentage losses were determined for Westminster and the City:[3]

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Year
City	71	58	43	25	12	12	14	16	25	40	62	83	38
Westminster	62	55	45	30	18	19	17	14	22	35	61	61	36

The percentage of sunshine in Birmingham compared with Oxford,

[1] British Association Meeting, Manchester, 1915. In Pittsburg the damage was estimated at £4 per head.

[2] *Journ. Soc. Chem. Ind.*, 1911, 1360.

[3] Rep. of London Fog Inquiry, 1902–3, *Meteorolog. Off. Publ.*, No. 160.

and in Glasgow and Manchester compared with Stonyhurst, is given as follows:

	Birmingham Oxford		Glasgow Stonyhurst		Manchester Stonyhurst	
	Winter	Summer	Winter	Summer	Winter	Summer
1881–5	—	—	64	81	—	—
1886–90	82	75	63	90	—	—
1891–5	71	71	72	89	—	—
1896–1900	73	72	79	73	62	90
1901–5	60	80	51	84	62	91
1906–10	59	82	57	94	63	92

The observations in the three cities were made in the suburbs and not in the worst parts.[1]

W. A. Bone estimates the quantity of soot and smoke emitted by domestic fires at 3·3 million tons, by factory furnaces at 6 million tons, in all about 10 million tons in the year. The domestic soot enhances winter fogs, and is more tarry and tenacious, and therefore more pernicious to vegetation. The flue smoke of factories, on the other hand, is probably more pernicious to the lungs (see p. 165).

The average yearly deposit at Leeds within the central 4 sq. mile area was given as 190 tons per sq. mile. The deposit at Old Street, E.C., was estimated at 650 tons per sq. mile, and at Buckingham Gate 500 tons, against 195 at Cheam, Surrey, and there was six times as much soot and sulphate of ammonium in the London deposit as in that at Cheam (J. S. Owens). The fall of soot is estimated at 89 tons per sq. mile per month at Rochdale, 53 at Newcastle-on-Tyne, 6½ at Malvern. The suspended sooty material in the air can be continuously recorded by filtration of measured quantities of air through a measured area of filter paper and testing the discoloration of the paper against a standard.[2]

J. B. Cohen found in Manchester the number of milligrams of SO_3 per 100 cubic feet of air increased from 1·8 in September to 32·2 in a December fog.

The researches of Aitken show that an atmosphere free from, or even relatively free from, dust and acid particles is scarcely able to produce fog or mist even when cooled below the dewpoint.

The employment of coke on small fires is much better than coal, but sulphur compounds are retained to some extent even in the best coke. The output of sulphuric acid from combustion of gas is probably not more than one-twentieth of that from coal.

Aitken [3] found the following number of particles round which water vapour condenses:

	No. per c.c.
4 ft. from floor of room in which gas was burning	1,860,000
Near ceiling	5,470,000
Air above Bunsen flame	30,000,000
On the shore of Loch	205–4,000
At the top of Ben Nevis	335–473
In open air of London or Paris, many hundreds of thousands.	

Fog is made dense by water deposited on particles of dust, soot, or acid.

[1] R. Lempert, *Report Coal Smoke Abatement Soc. Conference*, 1912, 23.

[2] W. Thomson, *J. Chem. Technol.*, July 1915; *5th Rep. Atm. Pollution Comm. Meteorol. Office*, 1919; J. S. Owens, *Roy. Meteorol. Soc.*, 1918, **44**, No. 187.

[3] *Nature*, March 1, 1888.

Be it noted that Aitken's method counts every particle, however small, or ionized molecular aggregate which collects its droplet of moisture.

There is a great diminution of dust after rain, e.g. 100,000 per c.c. on the top of the Eiffel Tower, Paris, are reduced to 300 after a shower. The raindrops pull down with them the pure upper air strata. The rain storms which sweep the cities have a most important cleansing action, and the weather, condemned as inclement and unpleasant, is an agent of good health, cleaning air, streets, and drains.

The choking of the stomata by tarry matter, and the presence of free acids in the air, and deposited on the soil, have a most detrimental effect on vegetation, and lessen the pleasure and health derived from the cultivation of gardens and allotments, and the enjoyment and use of the public parks.

In Leeds 80 per cent. of the stomata of leaves of conifers are frequently found choked.

In milligram's per sq. metre of leaf surface there was deposited on aucuba leaves (Cohen) :

In Manchester City	728	holly
	568	
	833	
In the suburban parks	374	
	194	
	131	

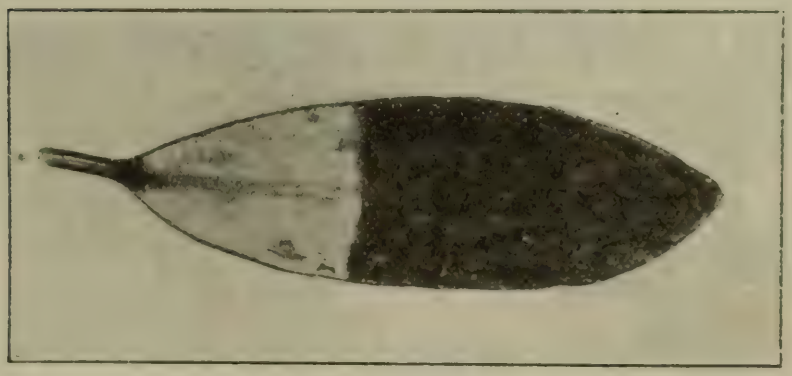


Fig. 19. Photograph of leaf showing soot half removed. J. B. Cohen.

The following values were obtained in Leeds and district :

	Annual deposit of soot	Assimilatory powers of laurel leaves
Westmeed Lane	147 lb. per acre	100
Headingley Hill	273	53
University	399	42
City Square	849	12

The laurel plants in the more polluted areas were all stunted in size. In Hunslet, one of the most polluted areas, no laurels survived. The majority of the samples of rain collected at the stations were decidedly acid to methyl orange, indicating the presence of free mineral acid. Alkaline impurities were issued by some furnaces.

The following estimates were made :

	Total deposited matter	Total sulphur compound	Free acid
Leeds Forge	1,886 lb. per acre	157 lb. per acre	30 lb. per acre
Hunslet	1,565 ,,	209 ,,	90 ,,

Ash-trees shed their leaves 4, 6, and even 8 weeks earlier in the most polluted than in the purer parts of Leeds. The leaf as the factory of the plant is closed for 6 weeks of its working year, and works at less than half its normal rate in the polluted district.

The destruction of vegetation is of the greatest importance to health, not only from the aesthetic side, but from the fact that the supply of fresh green foods and milk of cows fed on such are the protective foods containing the essential food-accessory bodies. The want of these is one of the great causes of malnutrition in cities.

The evaporation of water is hindered, and this together with the radiative power of the fog particles and the cutting off of the radiant energy of the sun by smoke greatly increases the cold humidity of the climate, and impels the people indoors and to indoor occupations and pleasures in place of healthy exercise. The deleterious effects so produced are vastly more important than the blackening of the lung by inhaled soot.

Men live long who work in the clean air of the fields. No less imperative than a clean water supply or proper drainage system is a clean air supply.

The investigations made by Ascher, in Königsberg, in 1905, upon the relation between the purity of the air supply and respiratory diseases led him to this conclusion, and to the founding, in 1906, of a Kommission zur Bekämpfung des Rauches. Similar evidence is to be drawn from the death-rates during long-continued fogs in London. For a period of fog between Jan. 24 and Feb. 7, 1880, the death-rate rose from 27 to 48. In 1892 after a fortnight of fog it rose to 42.

In 1909 Glasgow experienced fogs which appear to demonstrate clearly their effect in increasing the death-rate from respiratory disease, the death-rate being compared with seven other towns in Scotland where the conditions of life are similar, and where the temperature was the same, but where, owing to their geographical positions, they had not fog when Glasgow had it.[1]

TABLE III.—*Deaths from Bronchial Diseases* (*Bronchitis, Pneumonia, Pleurisy*).

1909–10	Glasgow Number	Glasgow Rate	7 other towns Number	7 other towns Rate	Mean City temp.	Weather conditions
October (weekly average)	35	2·1	31	1·6	49·2° F.	Clear to dull.
Week ending Nov. 6	61	3·6	44	2·3	45·0	3 days fog.
,, ,, ,, 13	75	4·5	39	2·0	44·0	Dull, slight fog.
,, ,, ,, 20	138	8·3	55	2·9	30·7	5 days dense fog.
,, ,, ,, 27	233	13·9	63	3·3	40·3	Hazy.
,, ,, Dec. 4	171	10·2	93	4·8	42·3	Hazy or wet.
,, ,, ,, 11	198	11·8	76	4·0	37·0	4 days fog.
,, ,, ,, 18	137	8·2	78	4·0	38·5	Slight haze.
,, ,, ,, 25	95	5·7	76	4·0	31·9	Slight fog and clear.
,, ,, Jan. 1	93	5·6	88	4·6	43·1	Wet or clear, 1 day fog.

In a discussion on the value of the purity of the air we breathe held by the Scientific and Medical Societies of Winnipeg (January 7, 1913), Dr. Bond said: 'Here we are much blessed with a bright keen

[1] *Rep. Intern. Smoke Abatement Exhibition*, 1912.

atmosphere, and that with an unlimited amount of motion. Recently, however, we have had a week of foggy, breezeless weather. All the material that was poured out from men and other animals and exhalations from chimneys were not removed. We know what has been the result on the people of this city. There has been more trouble in the way of bad attacks of bronchitis and pneumonia and bad colds than we should have had throughout the whole of our Manitoba winter.'

Professor W. A. Bone says the chemist is naturally amazed and indignant that the evil of coal smoke should have been complacently tolerated so long. 'It is an index of the *laisser-faire* methods of our so-called governing classes whose attitude towards reforming has been to do just enough to keep the mob quiet. And the mob in our manufacturing areas has grown so accustomed to dirt and squalor that it habitually accepts the outpouring of black smoke from factory chimneys as part of the natural order, and an infallible sign of national prosperity.'

The securing of a national economic use of coal and the prevention of smoke are one and the same problem. The waste of coal fouls the earth with ashes and soot, the sky with smoke, destroys beauty, dirties houses, makes cities dismal, causes vast expenditure in cleaning, painting, rehanging, and furnishing. Fog caused by smoke wastes artificial light and impedes transport of people and goods. The measurable damage produced by smoke in London has been estimated at four million pounds a year. The loss of health and happiness is immeasurable.

There are some two million tons of nitrogen in the coal used annually which might be drawn on for fertilisers and used to replace the nitrates imported from Chili.

So inefficient are the means of converting the thermal energy of coal into power, steam or electric, that Professor W. A. Bone considers only 4 per cent. of the energy of the coal is utilized. Far less is utilized for lighting.

The average efficiency of the electric power stations is given by Sir Dugald Clerk and other authorities as under 8 per cent., 92 per cent. of the fuel value of coal being there wasted in the conversion of thermal into electric energy. The luminous efficiency of the metal filament lamp is given as 5 per cent.; that is to say, only 5 per cent. of 8 per cent., say ½ per cent., of the fuel value of the coal used to produce it is converted into electric light. In the case of an oil lamp or candle the luminous efficiency is only 1–10 per cent. All the rest of the fuel value is wasted as heat. On the other hand, the luminous efficiency of the glowworm, or firefly, is 100 per cent.; none of the energy of the material oxidized to produce light in the glow organ is wasted as heat.

It is estimated that to produce one candle-power per hour by gas or electricity approximately the same amount of coal must be used, putting the gas-mantle against the metal filament lamp. But gas has from a national point of view an economic advantage, since from every pound of coal used to make gas half a pound of coke remains over, with a fuel value equal to that of half a pound of coal. There are also valuable by-products—tar, creosote, oil, benzol, the mother substances of dyes and explosives, ammonia, and the valuable fertiliser

ammonia sulphate. Taking the coke and other by-products into account, it appears that at least 10 per cent. of the thermal energy of the coal is secured when gas is used for lighting. There are, of course, advantages of convenience and life of filament lamp versus mantle to be considered, but the difference in coal economy is great.

A gas-mantle burns organic matter in the air of a room, it helps to heat a room, and maintains ventilation by the upflow of heated air. The slight production of sulphuric acid fumes from the gas-mantle is fixed by the lime of plaster walls and ceiling. Water which condenses on a bowl of ice in a gas-lit room is slightly acid, and this water is, owing to the acid, practically sterile; this is not the case when electric lights are used (S. Rideal). On the other hand, electric light does not damage books, pictures, &c., and heats the air less.

The modern radiant-heat gas fire is estimated to give 30 per cent. of the thermal energy of the coal which is used for producing the gas consumed, and then the coke and tar products are left over; the electric heater gives us 8 per cent. of the energy of the coal used for production of the electricity consumed by it; for heating and boiling water gas gives 25·30 per cent., electricity about 4 per cent.; for cooking by oven gas gives 4·9 per cent., electricity about 2 per cent. Thus, to use electricity generated from coal for heating is, as Lord Moulton recently said, 'a ghastly waste'.

Super-electric stations may, it is estimated, double the efficiency of production of electricity, but there is no prospect of the economical use of coal at such stations approaching that at gas-works. Gas-works and electric power stations must be run in combination. By carbonizing all coal at gas-works, and using the coke for generating electric power and lighting, the gas for heating, lighting, and power, and securing the valuable by-products, an enormous saving of coal can be effected, and the total utilizable energy of the coal raised to 20 per cent., even to 30 per cent., the nation's coal supply made five, even seven times greater, without an extra ton being dug! At the same time, the sky and the cities can be made clean, and health, happiness, and human efficiency vastly increased. The present crisis will be a blessing in disguise if it forces the nation to use gas and coke in place of raw coal.

By low temperature carbonization of certain coals a smokeless semi-coke can be obtained for fuel and 'light oil' which can replace petrol when the world's store of oil becomes exhausted—a contingency not remote, according to Professor Armstrong. The great need of the moment is to cheapen the production of gas and smokeless fuel. Coal is the cheapest source of heat, but the loss of health, &c., due to smoke pollution makes it, in truth, far the dearest. This fact requires general recognition.

Apart from the question of carbonizing all coal, and forbidding its use in the raw state, there are at present great sources of waste. For example, to bake the same amount of bread the least efficient bakeries use seven times more coal than the most efficient; the milk vendors use anything up to half a ton of coal to pasteurize 1,000 gallons of milk, the whole of which could be saved if milk were chilled by deep well water and cleanly handled. Growers of early 'luxury' tomatoes use twice as much coal per acre of glass as growers of 'food' tomatoes;

asylums wherein coal is carefully conserved by a special officer use one-fifth of the coal used by other asylums where there is lack of control; a war hospital equipped with an American central heating apparatus used seven times as much coal, and with no advantage to health, as one fitted with no such apparatus. By lessening the waste of water coal used for pumping can be saved. In every large industrial area there are enormous losses of thermal energy which could be saved by co-operative control.

Incomplete combustion is due to two main causes: (1) cooling of the vapour or gases below their ignition temperatures (1100–1250° F.) before they are completely burnt, (2) insufficient air at the right place and time or bad mixing of air and gases. Intelligent stoking in small quantities at a time is then of very great importance in preventing waste and smoke. In a prize competition on hand-firing of boilers at Sheffield there was a gain of 18 per cent. in boiler efficiency between the best and the worst man.

An intelligent fireman is worth good pay, and many waste more than their wages in ignorant or careless handling of their furnaces. Dark, hot, ill-ventilated basements do not attract men of intelligence.

Contrasting two firemen on a locomotive on a trial run, the more capable saved $43\frac{3}{4}$ per cent. of coal with plenty of steam and no smoke.[1] While the stoker, then, is all important in smoke prevention it does not appear that he personally profits by his better work.

Besides getting rid of smoke the substitution of gas furnaces for coal in factories gives the manufacturer great economy in floor space and labour (conveyance, storage of fuel, stoking, removal of ashes), enables him to place his furnaces in any convenient position, gives him a uniform calorific value, and cleanliness.

[1] *Rep. Internal Smoke Abatement Exhibition*, 1912, 102.

CHAPTER III

Dust

The methods hitherto used for determining the dustiness of air have been critically examined by G. T. Palmer and others on behalf of the New York Commission on Ventilation.[1]

They include :

1. Exposing oiled plates to the air for a definite time, washing the plates with oil, and comparing the colour with dust and oil standards. This method is mainly applicable to soot (Liefmann).

2. Revolving a paddle-wheel covered with syrup in air and noting the resulting discoloration of the white blades.

3. Drawing a measured amount of air to be tested into a small chamber containing dust-free air, and by rarifying the air within this chamber causing a deposit of moisture on each dust particle ; the intensity of the fog produced is compared with translucent glass standards, or the particles allowed to fall on a glass slide and counted (Aitken). Not only particles but ions attached to aggregates of molecules act as foci of condensation.

4. Suspending a container of pure water in the air for several weeks and then filtering off, drying, and weighing the accumulated solids.

5. Filtering a measured amount of air through collodion wool. dissolving this in ether, and counting the particles in a small measured quantity of the liquid (Hahn), or filtering through sugar and dissolving in water (Winslow and others).

6. Filtering a measured amount of air through a weighed paper or cotton-wool filter and noting the increase in weight—the taking up of moisture by the paper or wool may vitiate this method. A thin paper thimble gives accurate results if a large volume of air, e.g. 1,000 cubic feet, is drawn through it.

7. Drawing a measured quantity of air through a water spray and counting the particles in a small measured quantity of the water, or evaporating off the water and weighing the residue.

The air is drawn through a U-tube containing the water, and the water is thrown by the current of air into a spray in a conical glass vessel, which adjoins the U-tube. A fan driven by an electric motor completes the apparatus, which is portable and convenient (G. T. Palmer). Fig. 20.

8. Passing a measured volume of air through a cylinder in which is inserted a shallow dish covered with glycerine (Graham Rogers). As the air stream is propelled against the dish, the heavier particles are caught on the glycerine, the air and lighter particles passing on round the edge of the dish. The particles on the dish are counted under the

[1] G. T. Palmer, L. V. Coleman, and H. C. Ward, *Am. J. Pub. Health*, 1916, **6**, 1049.

microscope. This gives the larger particles only and is an inefficient method.

9. Dust blown up so as to make a cloud is highly charged and can be precipitated on to a high tension plate, e.g. using a potential of 40,000 volts. Gas generated from coke used in Bessemer steel furnaces is thus purified and used for combustion and generation of power. The application of this principle to the sampling of dust has been studied by Dr. Macmillan (New York City Health Department).

Counting particles of dust in a beam of light (the ultra-microscopic method) has not so far proved a useful method.

Estimating the dust content by colour or discoloration methods is suitable for soot or smoke, but not suitable in comparing dust from factory processes.

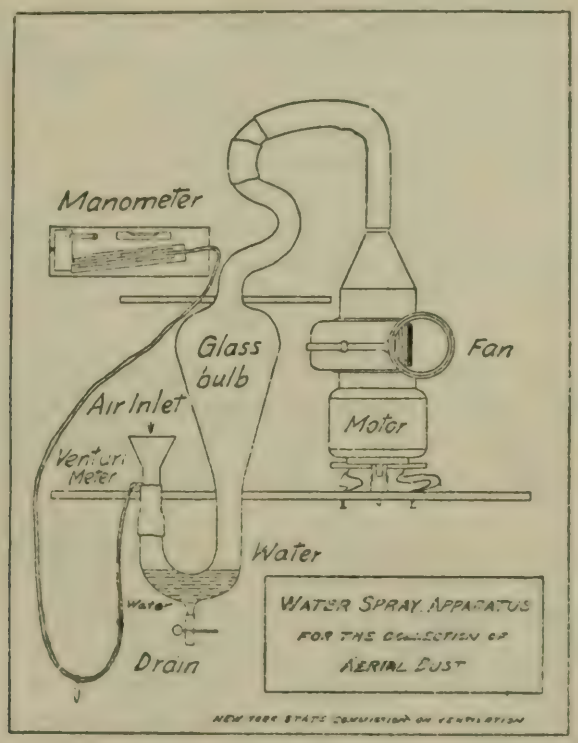


Fig. 20. G. T. Palmer.

Weighing is a more exact method, but owing to the extreme lightness of dust large volumes of air have to be sampled, and this method gives no information as to the size and character of the particle. Counting with the aid of the microscope gives this information, but is subject to serious sources of error. It is only possible to count the dust in a very small portion of the sample, and the conversion factor magnifies error. Irregularities on the glass slide may not be differentiable from dust particles.

If large enough samples of air are passed through, the efficiency of the sugar filter and the water washing methods is about the same, but while the sugar filter requires nearly seven hours to filter 100 cubic feet of air—an amount necessary for weighing purposes (unless the air is very dusty), the air washer passes this amount in about 30 minutes, as it offers so much less resistance.

In the sugar filter method the sugar, with its contained dust, is emptied into a test tube, and the sugar dissolved in 10 c.c. of water. One c.c. is then transferred to the counting cell, 50 mm. by 20 mm. by 1 mm. deep, and 10 areas counted by means of a micrometer eye-piece. No allowance need be made for dust in white sugar. For counting bacteria a sterilized sand filter is used in place of sugar, the sand shaken with 10 c.c. of sterile water, and the water examined for bacteria in the usual way.

In the case of the water spray apparatus 40 c.c. of water are placed in the bulb, and the loss by evaporation made up from time to time in order to keep the air-flow constant.

The water with suspended dust is drained into a 100 c.c. flask, and the bulb rinsed several times with distilled water and the rinsings added. The final volume is made up to 100 c.c., and one c.c. of this counted in the cell as above.

To make the error as small as possible a relatively large volume of air is sampled, and both weighing and counting employed. Both are needed for the interpretation of air dustiness. The fluff of bedding, carpets, &c., has a high count, but weighs little ; an atmosphere laden with iron or marble dust gives a much lower count and larger weight.

200 to 400 cubic feet of air should be drawn through the water spray (100 cubic feet suffices for air visibly dusty) at a rate not exceeding 5 cubic feet per min. Weighing should be carried out to $\frac{1}{10}$th milligram.

The cell should stand still for five minutes before counting ; two distinct c.c. of the sample should be counted ; a quarter field should be counted at five points in the cell, at the centre and near the four corners, to minimize the inclination to seek out the more crowded areas.

Sampling the air at the air-inlets of commercial buildings in Boston, G. C. and M. C. Whipple [1] determined by microscopical counts the number of dust particles. The range was from 100,000 to nearly 1,000,000 per cubic feet. With active wind movement the numbers are probably much higher. The number of particles inhaled with 100,000 per cubic feet of air would be a million and a half per hour. The vast number of particles are very small, averaging 0·003 millimeter in diameter.

The air sampled on the roof showed lower dust counts than that in the streets.

These weights of dust in grammes per million litres are given as recorded by different observers : [2]

TABLE IV

	Country	City Street	Subway	Hat fur	Pearl Button
Paper thimble filter	0·06	2·5	—	—	—
Water spray method	0·1	1·1	1·7-3·0	2·0-5·0	0·2
Sugar filter	—	0·4-1·8	2·0-7·0	—	—
Cotton wool	—	—	—	—	—

[1] *Am. J. of Pub. Health*, 1913. 3. 1139.

[2] For references see G. T. Palmer, &c., cited above.

Rag	Mine Rock	Pottery	Lead
—	—	—	—
3.0–8.0	—	—	—
—	6.0–80.0	—	—
—	—	6.0–60.0	1.0–5.6

For the country the figure may be taken as less than 0·1, for city street about 1·0, for industrial processes from 1·0 to 100. The following figures are of interest:

TABLE V

	Count per c. ft.[1]	Weight grm. per million litres
Out of doors, street level	118,000	1·1
Wedworth, 10th floor	72,000	0·3
Building, 58th floor	23,000	0·17
Business office	128,000–172,000	0·2–0·4
Department store, basement	94,000–118,000	0·2–0·9
Pearl button factory	72,000–139,000	0·2
School luncheon-room [a]	258,000	1·8
Ditto [b]	97,000	0·3
Ditto [c]	1,090,000	2·0
Marble cutting shop [d]	590,000–691,000	1·9–2·4
Subway platform	1,130,000–2,320,000	1·7–3·0
Iron grinding room	14,800,000–48,700,000	4·4–7·5
Hat fur factory	435,000–701,000	1·9–4·7
Rag sorting shop	215,000–568,000	7·9–3·3
Mattress renovating shop	1,530,000–7,140,000	2·4–22·2
Feather drying with starch [e]	160,000–867,000	27·9–14·7

[a] 500–1,500 pupils present.
[b] Few present; windows open.
[c] Janitor sweeping.
[d] Compressed air chisels used.
[e] Very hot dry room; feathers packed in starch are beaten by machinery to remove starch.

G. T. Palmer and his co-workers classify four sizes of dust:

TABLE VI.—*Classification of Dust Particles from Different Environments by sizes. Per cent. of total count*

Source	Classes 1.	2.	3.	4.	5.	Comment
Approximate area—sq. mm.	0·04	0·01	0·004	0·0001	Too fine to count	
Outdoors, uptown	0	0	0·4	99·6	+	Very small.
Outdoors, downtown	0	0·02	2·58	97·4	+	Large.
Business office	0	0	0·85	99·15	±	Very small.
Dept. store basement	0	0	2·	98·	−	Lint. Irregular.
Pearl button factory	0	0	3·6	96·4	−	Regular. Small.
School lunch-room (D)	0	0	2·5	97·5	+	Small.
School lunch-room (E)	0	0·05	1·6	98·35	+	All sizes and shapes.
Marble polishing room	0	0	0·5	99·50	+	Large.
Subway station	0	0·02	0·5	99·48	−	Small.
Iron grinding room	0	0·04	0·71	99·25	+	Small.
Hat fur factory	0	0·08	1·68	98·14	+	Small.
Rag sorting shop	0·02	0·27	3·83	95·88	+	Long fibres.
Mattress renovating room	0·01	0·10	1·06	98·83	+	All sizes.
Starch room	0	0	26·	74·	−	Regular. Large.

[1] These figures must be multiplied roughly by 35 to convert into the number of dust particles per c. metre.

A rag sorting room appears to the eye a very dusty place, and the weights of dust vary from 3·3 to 7·9 gr. per million litres, yet the counts only run from 200,000 to 600,000. On the other hand, an iron grinding room seems less dusty to the eye and nose, yet while the weights are about the same the counts run from 3 to 50 million particles.

In polishing and grinding factories counts even up to 159 million dust particles per c. ft. of air have been found; a worker might easily inhale half a gramme of dust in a day.[1]

Light coloured dusts in a dark tinted room make the air appear dustier than dark coloured dusts.

In the pearl grinding room the wetting of wheels and local exhaust system kept down the dust except in the immediate range of the wheel.

Dust extractors in factories are commonly found to be wrongly designed, inefficient, and out of action. Winslow has given standards for measuring their efficiency[2] by means of the apparatus shown in Fig. 20.

Street dust contains soot and flue dust, rock dust from street paving, silica dust from granite and flint, dust from garbage and decaying leaves, manure of horses, dung of dogs, fragments of insects and their eggs, spores of moulds, micro-organisms, and pollen of plants, dust worn off boots and clothes. Road-sweepers stir it up, the wind on dry days drives it in clouds.

People fear the smell of sewer gas, revolt at dirty linen, unclean cups and plates, soiled food, impure water, but breathe into their lungs without comment the dust of the streets. Motor-cars have vastly increased the dust nuisance, acting as dust squirts, impelling into the air not only the dried filth of the streets but the dust from granite or flint or tarred surface. The law forbids a man to strike his neighbour, but he may assault the delicate structure of his neighbour's lung with dust.

The dust destroys the beauty of foliage along the roads, soils and damages houses and furniture contiguous to roads, pollutes food, soils clothes and the skin, and, causing discomfort, impels people from exercise in the open air.

The housewife or maid's activity with broom and brush fills the air of the house with dust which if left lying, or wet cleaned, or taken up with a vacuum cleaner, would not pollute the air. The effect of dry brushing and of people tramping on dry dusty surfaces is to increase enormously the dust in the air. Dust pervades every nook and cranny of a house, the sunbeam shows it dancing in the air, and the polished surface of furniture shows a film of dust every morning. Every current in the house carries it along. The wind blows in dust from the street, the boots bring it in, fluff worn off clothes and hangings, and epidermic scales off the skin, add to it. Southerly winds are more apt to bring dust and haze, while northerly and westerly winds bring pure air from off the ocean and from high altitudes. After desert sand-storms the faeces may be so gritty as to interfere with the

[1] Winslow, Greenburg, and Greenberg, *Public Health Rep. U.S.A.* Reprint, No. 530.
[2] *Publ. Health Rep. U.S.A.* Reprint, No. 509.

use of a cover slip in making a microscopical examination. (A. Balfour).

Coarse dust falls quickly, very fine dust may take hours to fall in a room, and the very finest may float for days. Dust remains suspended longer in dry than in moist air.

Tarring a road may reduce the street dust which enters a house 98 per cent. Side-walks require to be wet cleaned, not dry brushed. The rain keeps down the dust and purifies the air of cities enormously.

A rubber mat, the surface of which is wiped with a lightly oiled cloth, will catch boot dust far better than a fibre mat. The oiling of hardwood and polished floors renders them dust proof, and an oiled mop cleans them effectually. The vacuum cleaner is far the cleanest method of removing dust from rugs, wall-papers, hangings, and bedding.

Dust should be regarded in the same light as the fly and smoke nuisance; all three can be enormously reduced if the community will only see to it. Exposure to several kinds of dust greatly increases the incidence of bronchitis and pneumonia, and causes chronic asthma. Silica dust is a cause of phthisis, lead dust of lead poisoning.

The history of medical knowledge concerning the influence of dust on health (recognized by Pliny) is fully treated by E. L. Collis.[1]

'Owing to the dust in mines in the Carpathian mountains there are women who have married seven husbands, says a mediaeval writer.'

'The pounding of dry flints kills any worker in two years, says a patentee of a wet process in 1713.'

'Out of hemp and flax there flies a foul, mischievous powder that makes way for asthma.'

'The poor people that comb silk cakes are troubled with a vehement cough and a great difficulty of breathing, and few live to old age.'

'Those who sift corn are so plagued with this powder and dust . . . that almost all are short-breathed and cachectic, and seldom live to be old; nay, they are apt to be seized with an orthopnoea and at last with a dropsy.'

In modern terms they suffer from dust bronchitis, emphysema, dilated heart, and failure of the circulation.

Dust of every kind irritates, but not in equal degree, 'bricklayers and plasterer's labourers, like asses, never die.'

While masons who chip sandstone generally die before 40, those who work in limestone are sensible of no inconvenience.

H. H. Greenhow[2] established the influence of occupation on health, and throughout his reports runs as a theme the influence of dust in causing pulmonary disease. He found carding-room operatives and flax-hecklers asthmatical, the older men short-breathed with rounded shoulders, emaciated frames, prominent eyes, and laborious wheezing respiration.

Goadly showed that lead poisoning is caused one hundred times more easily by inhaling lead dust than by eating it.[3]

The bacterial contamination of the respiratory tract of people and

[1] Milroy Lectures, *Public Health*, 1915, **28**, 252 and 292.
[2] *Reports of Medical Officer to Privy Council*, 1860–2, cit. after Collis.
[3] *Lead Poisoning*, Arnold, 1912.

food exposed in shops, stalls, and larders, is enormous in dusty crowded places. The following figures are illustrative :

Sartory and Langlois found 42,000 bacteria per cubic metre of air in the picture galleries of the Salon on Saturday, and on Sundays 3,240,000 at 3 p.m , and 5,000,000 at 4 p.m.

In the staircase of a house there were 750 per cubic metre before sweeping, and after 10 minutes' brushing of the carpet, 410,000.

In the case of a stone staircase there were 140,000 after dry sweeping, and only 26,000 when the sweeping was preceded by abundant sprinkling with water.

At an underground station there were 410 at 9 a.m., at 11 a.m. 68,000, and after dry sweeping of the platform 210,000.

In rooms of laundries where linen was sorted the average was 450,000.

In a factory where horsehair was treated as many as 13,000,000 have been counted ; and in workshops where feathers were cleaned or hides scraped as many as 30,000,000.

These authors say that tobacco smoke greatly reduces the bacteria in the mouth and so with snuff in the nose.

They advise that dry sweeping be put down, that workmen should not eat in workrooms, and that measures should be taken to ensure adequate aeration, wet cleaning, and open spaces.

Carnelly and Haldane found 91,000 bacteria per cubic metre in the cleaner, and 108,000 in the dirtier, elementary schools; 310,000 in a school open 20 years, and 38,000 in a school open less than 2 years ; with fan ventilation 3,000 in the cleaner and 30,000 in the dirtier.

C. E. A. Winslow and W. W. Brown found the number of bacteria developing at 20° C. from outdoor air in suburban districts (U.S.A.) is generally under 1,400 per cubic metre. Those developing at 37° C. are generally about half this number. The number of mouth streptococci is small.

The air of city streets shows slightly higher numbers and the same relations. The air of occupied rooms show much higher numbers and larger fluctuations.

Those developing at 20° C. may average 3,000, and may reach 20,000 in some offices. Those developing at 37° C. are no less numerous, sometimes more numerous. Mouth streptococci are much more abundant and afford a good measure of saliva spray pollution due to concentration of population in confined spaces.[1]

In contrast with crowded rooms the pure air of mid-ocean yielded only 4 or 5 bacteria in 10 cubic metres.[2] That on the summit of Mont Blanc 4 to 11 in one cubic metre ; as the altitude diminished during the descent the figure rose to 49.[3] Air taken at height of 1,000 metres above a town is remarkably pure, while the air taken in the Park at Montseuris was found to be 28 times less charged with bacteria than that in Paris. That taken on the top of the Panthéon in Paris was purer than that taken at ground level in the Park. At

[1] Saturated air holds bacteria in suspension far better than dry air ; a cold surface, through condensation, is infected more than a warm surface on exposure to such a suspension. Trillat et Mailein, *Compt. rend. Acad. d.s c.* 1920, **170**, 1291.

[2] *Traité de Bactériologie*, Miquel and Cambier, Paris, 1902, 915.

[3] Binot, *Compt. rend. Acad. d. sc.* 1902, **134**, 673.

a height of 4,000 metres the atmosphere is probably quite free from bacteria.

Clean fresh air, as free from dust and bacteria as country air, will come to be regarded as essential as a clean water supply. The one method open to all is that of flooding rooms with abundant fresh outside air.

Crowded buildings and dusty city streets often render it impossible in dry weather to secure clean air from outside. Just as rain washes the air clean, so can dusty smoke-fouled air be cleaned by washing; acid and ammoniacal vapours and smell can be removed as well as bacteria and dust.

Air is washed by being passed through a chamber in which there are intersecting water sprays, forming a curtain of fine droplets. The water, circulated by means of a pump, may be used over and over again for a considerable time. Sulphur acids in smoke-contaminated air, acting on copper sprays, result in forming a trace of copper sulphate in the water, which helps to keep the bacteria count in the water very low.

At the Y.M.C.A. Gymnasium, Springfield (U.S.A.), the washed air is recirculated so as to change the air in the room 5 or 6 times an hour. Nothing is done to remove carbonic acid. The recirculated air after washing is cleaner than unwashed outdoor air, but slightly less clean than washed outdoor air. When the outdoor temperature was 32° F. the saving in cost of heating was 40 per cent.

Two-thirds of suspended particles, dust bacteria, and moulds can be removed by air washing as practised—much greater efficiency might be reached.[1]

Air may be thus washed and sent in clean by fan circulation and then be at once fouled with dust raised from the floor and off the boots of people moving about within the room.

Be it noted the Japanese take their boots off indoors.

The bacteriological investigation, by M. H. Gordon,[2] of the House of Commons—where there is an upcast ventilation through the perforated floor of the chamber to the roof outlets—showed that the boots of the members brought in faecal pollution—probably horse dung. Such pollution must occur in street and room where dust is stirred. There was no evidence of particles of sputum being brought in on boots from the streets and pavements. Many experiments were made in the House of speaking after washing the mouth out with a culture of *Bacillus prodigiosus*. With the present system of ventilation culture plates placed on the benches which the speaker faced were not infected (W. Bulloch and Hill). The current of air carries the salivary droplets up to the roof outlets. The members, however, infect each other when crowded in lobbies or dining-rooms and talking closely together, and are infected in crowded trains, meetings, &c., so that no advantage is gained. On the other hand, there is disadvantage from the up-current which cools the members feet more than their heads and congests their noses.

The *Bacillus influenza* and the pneumococcus are quickly destroyed outside the body, and such catarrhal organisms are undoubtedly spread

[1] G. C. and M. C. Whipple, *Am. J. Pub. Health*, 1913, 3, 1139.
[2] *House of Commons Ventilation*, col. 3068.

directly by salivary spray passing from man to man. Bacteria that cause enteritis, typhoid fever, dysentery, &c., on the other hand remain virulent outside the body for some weeks; so too tubercle bacilli.

Apart from bacteria, certain dusts may have a chemical, and, if hard and sharp, a mechanical irritating effect on the respiratory membrane.

The evidence drawn from dusty trades shows that while the respiratory membrane has enormous powers of filtering off and cleaning up dust, and some dusts are harmless, others contribute greatly to pulmonary disease.

In the filtering of particles, e.g. poisonous fumes, by the nose or by a respirator (gas mask), assuming a given adhesive force between the particles and the filter, and that the particles pass through a devious and winding passage way, the number of impacts which a given particle will make will depend on the time the particle is in the filter. With a high rate of flow particles are carried through.

The deep breathing of hard work will therefore carry dust into the lungs, which would be caught in the larger air passages during shallow breathing.

If air is ionized by explosive and disruptive discharges particles become charged. If these settle on a filter they may build up a charged area and repel similarly charged particles. A filter may recover on rest owing to dissipation of the charge.

The efficacy of the air passages as a filter is such as to generally render the air from the depths of the lung sterile. Lister noticed that if the lung is punctured by a simple fracture of a rib, the blood effused in the pleural cavity undergoes no decomposition.

Tyndall showed the air breathed out from the lungs was optically dark, containing no ultra-microscopical particles. The breath must be explosive, as in coughing, sneezing, and speaking, to carry out particles from the respiratory membrane.

As shown by experiments on animals, rabbits, which breathe only through the nose, the mucous surfaces of the turbinate bones at first act as an effective filter when air, heavily laden with dust, is inhaled. Finally the membrane becomes covered with dust and can retain no more. The dust also impinges on the pharynx, which is irritated together with the nose.

Among the children in dusty regions of West Australia adenoids are very prevalent. A cycle ride along roads made dusty by motor-cars often produces an acute attack of coryza and conjunctivitis. Similarly the dust in cotton stripping rooms may give a visitor an attack of 'mill fever' some hours later.

Zinc fumes produce 'chills' in bronze and brass casters. Such workers suffer from bronchitis, pulmonary tuberculosis, dyspepsia, and few are found at work over forty years of age. Very fine dust passes down into the alveoli of the lung. Flint dust may form so fine a cloud that it can be poured like a heavy gas from one vessel into another.

The great majority of the silica particles found in the ash of the silicated lung have a diameter of less than 1 μ. They enter the lungs as a mist or fume and pass into the alveoli. No particles have been found in the lung-tissue which exceed 10 μ. The finest dust produced by blasting in the Rand mines averages 2·5 μ, and, it is said, there

may be as many as 86,000,000,000 particles in the air per cubic metre, just after blasting. The dust drifts through the mine, settles, and is stirred up again at each blasting.[1]

Dust of vegetable and animal origin may be better digested and dissolved, and soluble mineral dust dissolved by the respiratory membrane. Larger particles are entangled in mucus and swept away by ciliary action; smaller ones, when they reach the alveoli, are removed by phagocytes.

The mouth is not so well adapted as the nose for removing dust and micro-organisms. The mouth-breather has a chronically infected and obstructed nose. He is for both reasons more liable to bronchial catarrh.

The secretory action of the mucus glands, the cilia, and phagocytes clean the respiratory membrane from insoluble dust, but these defences may be overcome by exposure to dirty atmospheres; dust particles are then lodged in the perivascular and peribronchial lymphatics, and in the interalveolar tissue carried thither by phagocytes. Fibrosis of this tissue results and the particles are thus enclosed.

These parts become blackened in the lungs of city dwellers, together with the bronchial glands which form the terminal drainage system. The blackness in contrast with the pinky whiteness of the lungs of a new-born baby, or of sheep, is most striking: the bronchial glands may feel gritty to the knife.

Early fibrosis from silica dust is almost entirely perivascular and peribronchial; the alveolar walls are generally not thickened or reduced in size, but show signs of catarrh.

In the later stage there is thickening of the alveolar walls with reduction of the alveoli, of the interlobular septa, and of the pleura and subpleural tissue. The respiratory capacity is greatly reduced, and the circulation through the lungs obstructed. Catarrh is always present.

In the advanced stage of silicosis there is a generalized nodular fibrosis. Many hundreds of dense fibroid nodules are scattered through the lungs: where these join fibroid consolidation results, and the pleura is greatly thickened and generally adherent.

In the lungs of a tailor there was 0·9 grm. of silica per 100 grm. of dried lung; in a Rand gold miner 4·47 grm.

Beattie's researches,[2] confirming those of Arnold,[3] show that any kind of dust particles, except, perhaps, those that are soluble and chemically harmless, will cause inflammatory changes in the lungs of animals if enough are inhaled. Silicosis of the lungs resulted in rats which were kept for months in a Rand mine.

Bronchitis results from any stimulation of the bronchial mucosa which causes inflammatory hyperaemia and excessive exudation of mucus. High mortality from bronchitis occurs in masons who work sandstone; in Lancashire coal-miners owing to the dust of rock strata which lies between the coal seams; in potters owing to flint dust; in tin-workers owing to quartz dust.

Cotton, wool, flax, jute, hemp, slag-wool, glass, and emery also are pernicious.

[1] Watt, Irvine, &c., *Silicosis*, Pretoria, 1916.
[2] *First Rep. Mines Commission*, 1912, col. 6. 307.
[3] *Untersuch. über Staubinhalation*, etc., Leipzig, 1885.

All these dusts, says Collis, appear to be brittle and fine, insoluble in mucus, and non-colloidal in structure.

Dust blown up as cloud is highly charged. 'Acidic' substances, such as sand, are negatively, 'basic' substances such as flour and coal positively charged.[1] These properties may have bearing on the removal of dust in the lungs by phagocytosis.

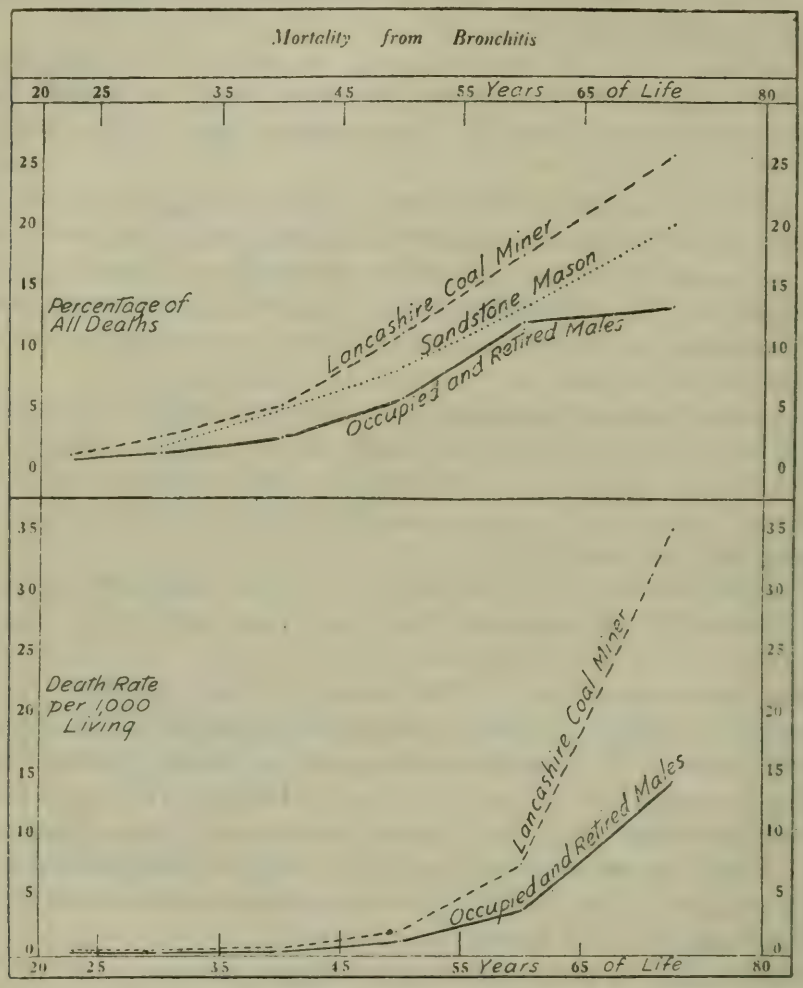


Fig. 21. E. L. Collis.

Collis gives the following figures for those exposed to emery and glass dust:

	Signs of pulmonary disease
Engaged in the dusty process	26 per cent.
Yard labourers and enginemen, &c., in the same works . .	8 per cent.

When from any cause pneumonia becomes epidemic, persons largely exposed to the inhalation of slag dust have a high fatality. So with dressers of iron-castings, and workers in quartz and silicious rocks.

Bronchitis and pneumonia run together.

The mortality from pneumonia of the natives employed in the Rand Mines, Ltd., was 2·65 per 1,000 in 1917 and 3·95 in 1918 (influenza epidemic); that of coloured males between 25–54, insured in the New York Metropolitan Insurance Co., 1·9 per 1,000.[2]

[1] Rudge, *Proc. Roy. Soc.*, 1914, **A. 90**, 256.

[2] Dublin, *Mortality Statistics of Insured*, New York, 1919.

The prevalence of respiratory disease among Lancashire miners probably depends on the amount of stone-dust present. Inhalation of coal-dust alone is not associated with bronchitis: no more is inhalation of dusts of limestone, pure clay, calcium sulphate, cement, bone, leather, horn, flour, and starch.

Workers in dusty atmospheres have the expansion of their lungs limited as age comes on by the growth of connective tissue which confines the dust accumulations; they tend to become 'asthmatic'—short

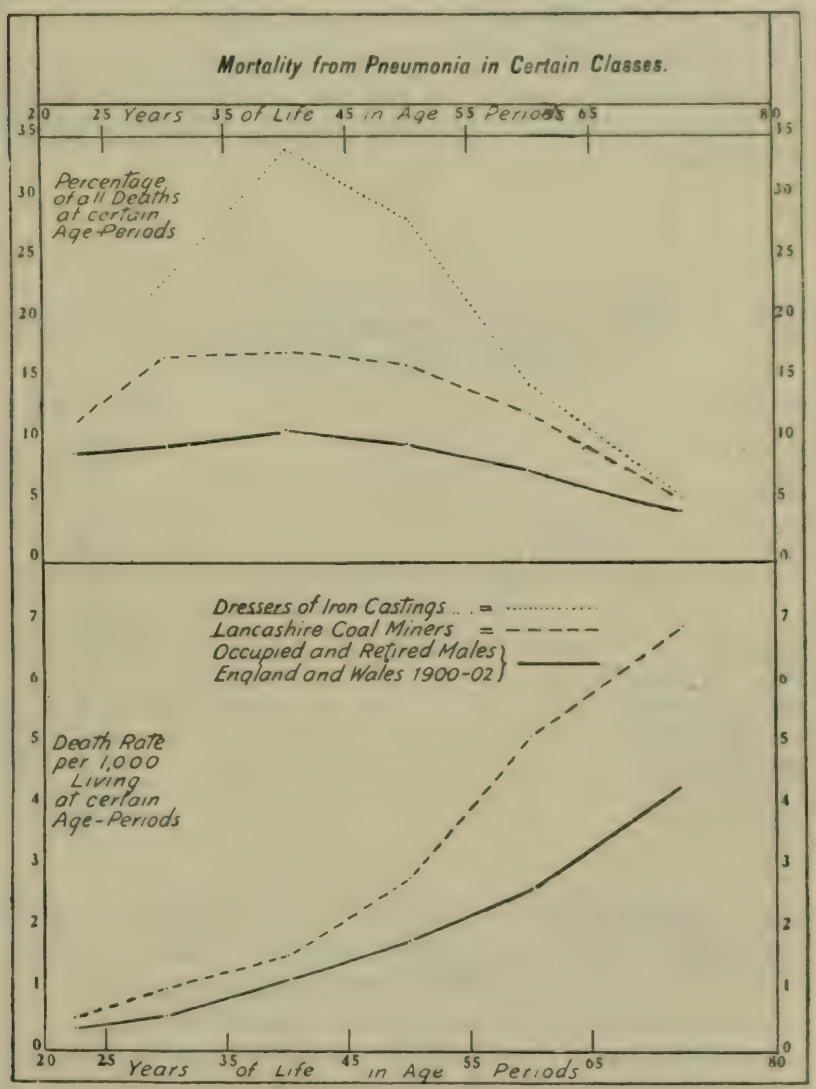


Fig. 22. E. L. Collis.

of breath. Fifty years ago 'asthma', says Collis, was common among coal miners, now with improved ventilation it is not so. Fumes of explosives, soot, and other products of combustion from naked lights, gaseous impurities, are minimized, and the opportunity of ascertaining which was the exciting cause has gone.

At Scranton, U.S.A., 'asthma' accounted, 1894–1904, for 7 per cent. of all deaths among coal miners, as compared with 1·6 per cent.

among all occupied males.[1] Miners' asthma is chronic bronchitis and emphysema.

It is suggested that coal dust is not only innocent but actually protective against phthisis.

Wainright and Nicholls give the following figures:[2]

	All lung diseases	Phthisis	Asthma	Pneumonia
Coal miners	100	14	29	42
Other occupied males	114	41	7	54

Haldane[3] says there is no evidence in the mortality returns for either young or old colliers that dust-inhalation has hitherto caused appreciable danger to life in British coal mining.

The peculiar harmful nature of uncombined silica dust, and innocent nature of coal and other dusts, in producing phthisis is shown by the following table:[4]

TABLE VII.—*Death-rates from Pulmonary Tuberculosis per* 1,000 *Living in Certain Dusty Occupations*

Occupation	Death-rate	
Brick maker	0·9	
Coal miner	1·0	
Mason (limestone)	1·4	
Iron-ore miner	1·5	
Slate worker	1·8	
All males (England and Wales) 15 years of age and over	2·1	
Potter	3·1	
Lead miner	3·9	
Cutler (Sheffield)	5·9	uncombined silica dust
Granite cutter (Aberdeen)	6·2	
Gold miner (Transvaal)	not less than 13·8	
Metal grinder (Sheffield)	15·2	
Mason (sandstone)	16·7	
Tin miner	17·6	
Ganister worker	22·3	
Flint-knapper	41·0	

Among gritstone workers the death-rate from phthisis was 13·7 per 1,000, limestone workers 1·52, coal miners 0·68. That of agriculturists in the gritstone area is 7·9, and 3·5 in the limestone area.

The effect of silica dust is even shown among them.

Among 70,000 men employed in silica dust trades Collis estimated the phthisis death-rate as five times the normal; there is no abnormal rate among cement, plaster of Paris, slag, wool, coal, and brick workers. The sulphur mine workers in Sicily suffer from pneumoconiosis and chronic lesions of the bronchi, but never, or hardly ever, from phthisis.[5]

The South African Government Commission in 1912 state that

[1] Wainwright and Nichols, *Am. J. M. Sci.*, 1905, **130**, 403.
[2] Cit. after F. Shufflebotnam, *Hygienic Aspect of Coal Mining*, Milroy Lectures, 1914.
[3] *Trans. Inst. Min. Engineers*, 1916–17, **51**, 469.
[4] E. L. Collis, Milroy Lectures, *Public Health*, 1915, **28**, 252 and 292.
[5] Carozzi, *Brit. M. J.*, Aug. 16, 1913.

out of 3,462 underground miners in the Transvaal who were specially examined and their history obtained, no fewer than 31·6 per cent. were affected with pneumoconiosis, and it was estimated that out of 11,400, the total number of miners in South Africa, the number affected could not be far from 3,600. Among machine drillers, 47·5 per cent. were

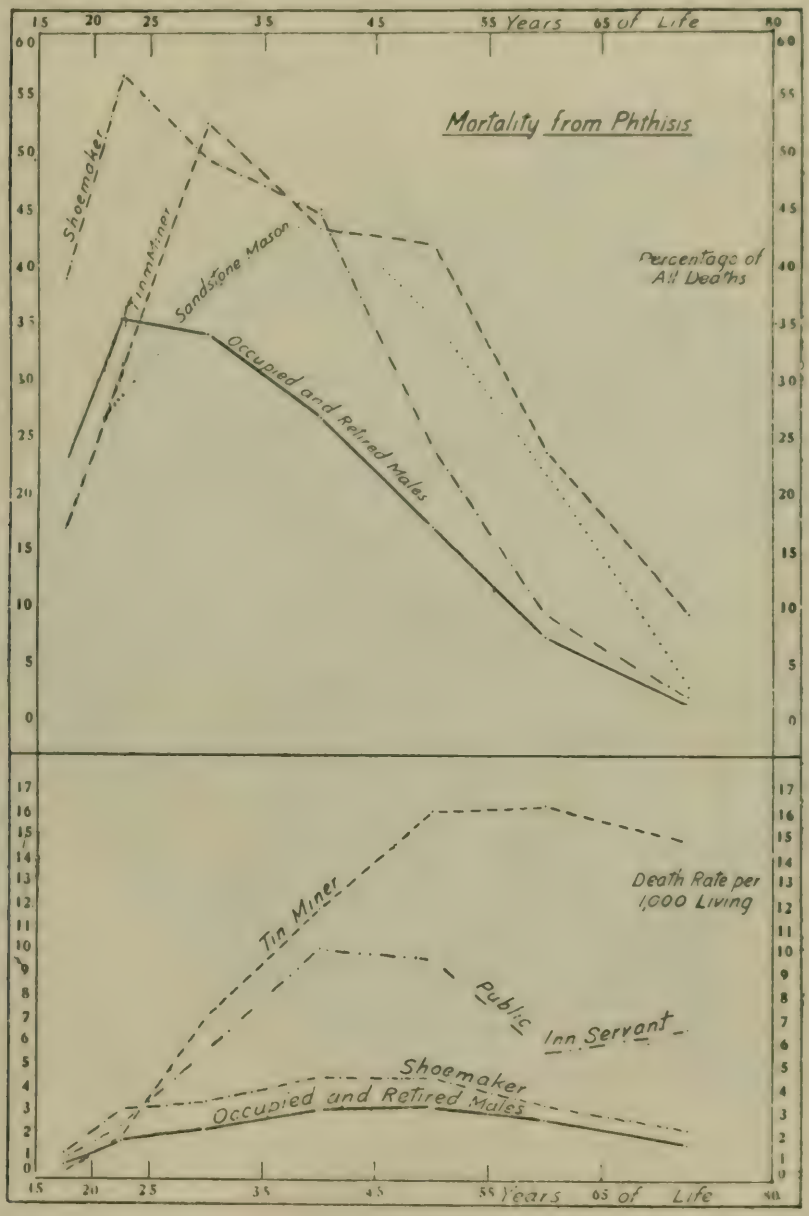


Fig. 23. E. L. Collis.

victims of the disease, while among those who had never done machine drilling 21 per cent. were affected. The inquiry was confined to European workers, and there was no investigation made into the health of natives or coloured workers. Although only 31·6 per cent. of the men examined were suffering from pneumoconiosis at the time of the examination, it was estimated that 90 per cent. of the workers would eventually contract the disease. It was found that amongst European

miners tuberculosis existed to the extent of 3·6 per cent. amongst those who were not affected by pneumoconiosis, and that of those affected by the latter disease 10 per cent. of them were tuberculous.

Collis pointed out that admixture of clay modifies the action of

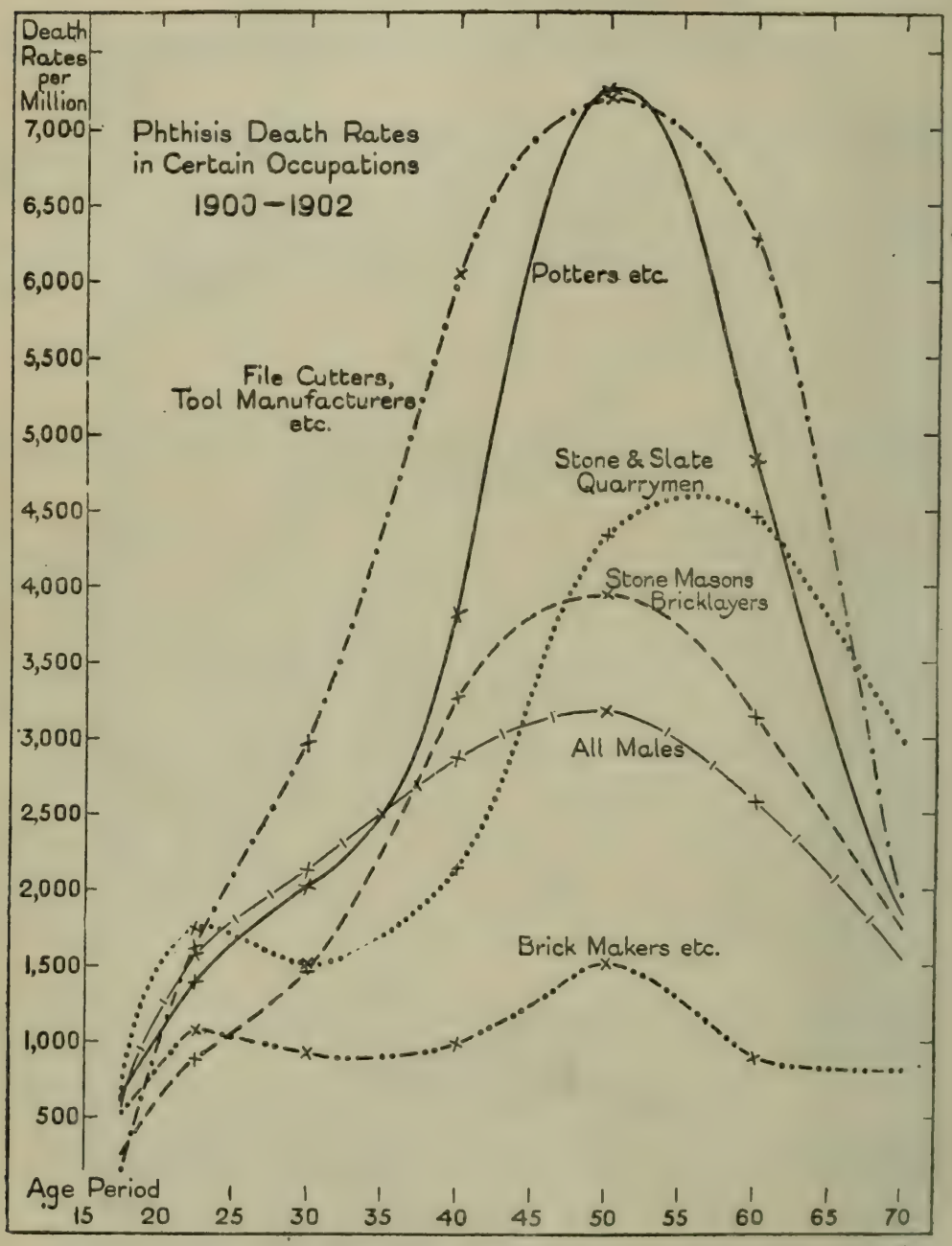


Fig. 24. J. Brownlee.

silica dust, preventing the latter causing phthisis. H. C. Ross suggested that the protecting action depends on organic constituents.

Mavrogordato [1] finds evidence that certain cells of those lining the alveoli act as phagocytes, and take up coal and other 'edible' dust

[1] *J. Hyg.*, 1918, **17**, 439.

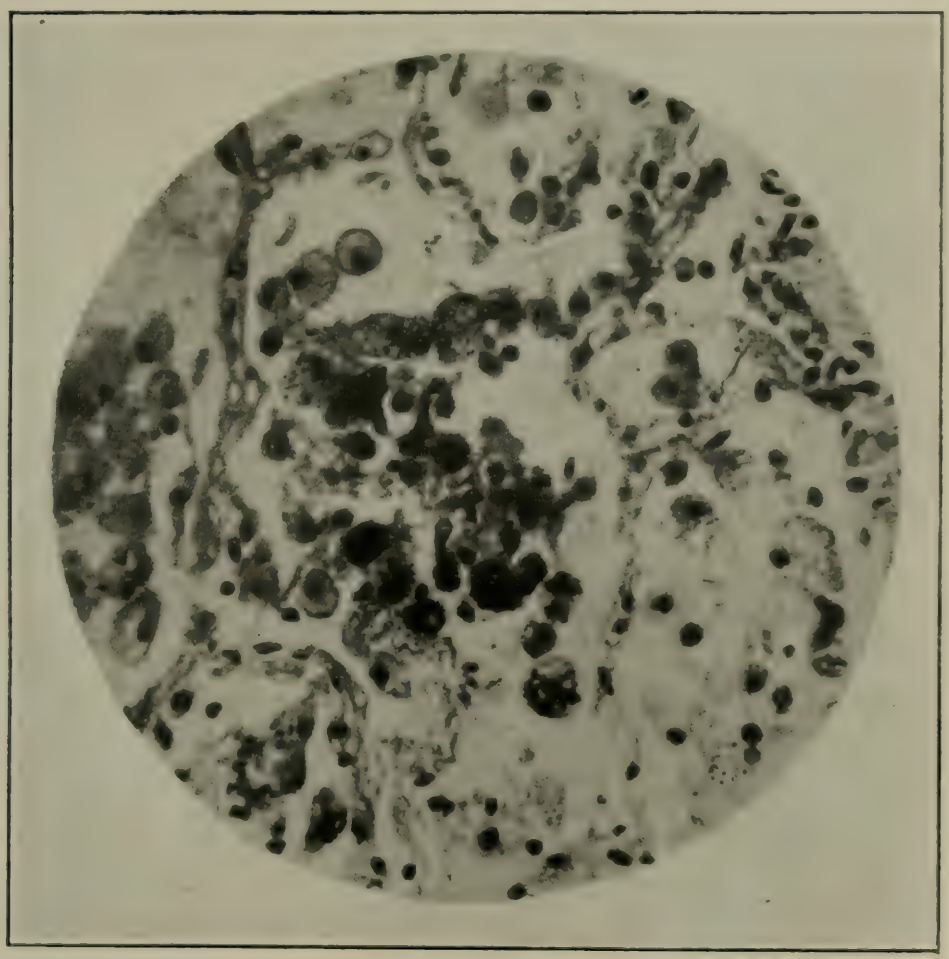


Fig. 25. Phagocytes in the alveoli of the lung loaded with coal dust. Watt, Irvine, &c.

particles, and, becoming detached, wander away with their dust loads either directly into the bronchial tubes or via the lymphatics to the lymphatic glandular tissue surrounding these tubes, and thence finally either into the bronchi or to the lymphatic glands at the roots of the lungs. The harmlessness of a dust depends on its power of exciting phagocytic action.

In the case of flint or quartzite dust nearly all that is inhaled remains *in situ*, the cells wander relatively little, and the lungs do not clean up. Flue dust, unlike raw coal, is not cleaned up in the lung, and is therefore a dangerous dust to inhale. Collis says neither glass nor emery dust causes phthisis, and great advantage has followed the replacing in Sheffield of gritstones by carborundum or emery wheels for sharpening tools.

If the ill-effects of quartz dust were due to the sharpness of the particles one would expect these to be in proportion to the amount inhaled, not that admixture of another dust, such as coal, would be protective. The particles of silica do not appear specially sharp or different from other very fine particles.

If it is not the hardness and sharpness of the particle that makes silica dust dangerous, the danger must arise either owing to its inert character, leading to its non-removal, or to chemical action. Flint or quartz when rubbed produces fire and a smell like burning cotton. Masons know that any rock that gives off this smell when worked is dangerous; they call it 'sulphur' (Collis). The fact may be recalled that water distilled in copper is poisonous to small forms of aquatic life, while water distilled in glass is not (Locke). The addition of a salt neutralizes the poisonous action of the copper. It is estimated that the copper in solution is not more than one part in 70,000. The insolubility of silica may not prevent its having similarly some chemical action.

Haldane lays stress on the power of a dust particle to absorb other substances which attract phagocytes. It is, he thinks, the inertness of silica dust in this respect which leads to its heaping up in the lung and so to fibrosis. He mentions quarries where hard stone, containing about 70 per cent. of silica, is worked and dusty crushers are in use, and much dust inhaled without any evidence of harm to the workers. A hale-looking old man who had been working for 30 years at dusty crushing mills, said the dust was not good to breathe as it had a very fattening effect!

In the Rand mines the rock is pure quartzite and phthisis is rampant. At the Mysore mine, on the other hand, there are quartzite veins, and the dust is mingled with other rock dust, and there is no excess of phthisis.

If coal dust be added to flint dust and animals inhale the mixture, the lungs are cleaned up by phagocytic action, and the deadly character of pure flint dust is set aside. It is suggested, then, that the Rand mines may be made safe by sprinkling the ways with a mixture of coal 30 per cent. and shale 70 per cent.

In the Nottingham district the coal dust is mixed with a great deal of silica dust from adjacent seams of rock. One sample showed as much as 70 per cent. The death-rate among the miners is at all ages up to 55 far below that of other employments, and even slightly

lower than that of farm labourers.[1] The conclusion is drawn, then, that it is safe to mix rock dust, such as shale containing silica, with the coal dust in the ways of other mines in order to remove the danger of coal dust explosions (Garforth's method). At least one-third of the weight of shale is fine crystalline particles of silica. The strength of most great mine explosions has been derived from coal dust, not from gas, and the poisonous fumes come mainly from imperfect combustion of coal dust. At Courrières, where 1,100 lives were lost, there was no fire-damp—the explosion was due to coal dust.

Ganister (about 97 per cent. SiO_2, 1–15 per cent. Al_2O_3) bonded with lime into bricks, provokes no cellular reaction, and the dust causes phthisis ; ganister bonded with fire-clay (60–69 per cent. SiO_2, 26–38 per cent. Al_2O_3) provokes cellular reaction, and the dust does not cause phthisis (E. L. Collis and W. S. Smith).[2]

By suitable choice of dust mixtures, then, it appears possible to stop the causation of silicosis of the lung and phthisis arising therefrom, and make unnecessary the wearing of respirators and other precautions taken to prevent inhalation of dust.

Water-sprays are now used, and with advantage, to keep down dust arising from rock-drills in the Rand and Cornish mines.

It is suggested that the sprinkling of the ways in these mines with coal and shale dust affords another means of preventing silicosis of the lung and miners' phthisis. Against this stands the fact recorded by Orenstein [3] that the incidence rate of miners' phthisis, as well as the mortality rate, is higher at the Witbank Colliery than on the gold mines of the central Rand Mines Group. As natives who show signs of silicosis are rejected on applying for employment at the colliery, it follows that early cases of silicosis progress in this coal mine, ' a fact which throws considerable doubt on the alleged efficacy of coal dust in arresting the development of silicosis '.

The next table compiled by Collis is of very great interest in showing the far greater incidence of deaths from respiratory disease in shut-up granite cutters' shops than in open-air sections of the works. The dust is greatly intensified in the shut-up shops and the defensive mechanism lowered by the warmer stagnant air ; see *Medical Research Committee, Special Report Series*, No. 32, p. 150 seq.

TABLE VIII.—*Aberdeen Granite Cutters*

Causes of deaths	Class of employment	Percentage of all deaths	Median age at death
Tuberculosis	Building section (open to the air)	25·0	54
	Monumental section (closed in)	38·0	46–47
Respiratory diseases other than tuberculosis	Building section (open to the air)	13·8	60
	Monumental section (closed in)	16·3	48–49
All other diseases	Building section (open to the air)	61·2	56–57
	Monumental section (closed in)	45·7	43–44

Compiled from lists of deaths published annually in the *United Operative Masons'* and *Granite Cutters' Journal*, published in Aberdeen.

[1] J. Haldane, *Inst. Min. Engineers*, June 13, 1918.
[2] *Home Office Report on Manufacture of Silica Bricks*, 1917.
[3] *Rand Mines Ltd., Dept. of Sanitation Rep.*, Nov. 4, 1918.

Of dangerous trade dusts which produce poisoning one may mention the explosive tri-nitro-toluol, which enters the body mainly through the skin. (B. Moore and Webster.) Suitable methods of cleanliness, mechanical handling, and dust extraction allowed the danger of such a poisonous dust to be overcome.

It is worth noting that any fine cloud of vegetable dust, such as flour, can act as an explosive if fired no less than coal dust.

CHAPTER IV

Heat Stagnation and Fitness.

More than thirty-five years ago Hermans[1] pointed out the insufficiency of the chemical theory, and suggested that the results of bad ventilation were due to heat stagnation, the symptoms felt in crowded rooms—oppression, headache, sickness, and faintness—resembling those experienced out of doors on excessively hot humid days. He pointed out that the heat and moisture of the air increase very greatly in crowds jammed together, while clothes increase 13 to 14 per cent. in weight, their wetness increasing the unpleasant feelings and the danger of chill on coming out.

Under the direction of Flügge[2] a series of convincing experiments were carried out on this question by Heymann, Paul, Ercklentz, &c. Men were shut in a cabinet of 3 cm. capacity for periods up to four hours and until the CO_2 percentage rose to 1·0–1·5. No discomfort was felt or any lessening of efficiency in the carrying out of graphic or mental tests, so long as the temperature and moisture of the air were kept low.

In one experiment the temperature of the chamber rose to 24° C., the relative humidity was 89 per cent., the CO_2 1·2 per cent. The man inside was very uncomfortable. No discomfort was felt by those outside on breathing through a tube the air inside.

In another case the temperature of the chamber was 30·2° C., the relative humidity 87 per cent., the CO_2 1·1 per cent. The man inside was not relieved of his discomfort by breathing through a tube the cool outside air, but his symptoms were allayed by a fan inside the chamber. When the chamber was cooled to 17° C., no discomfort was felt, although the CO_2 rose to 1·6 per cent. Those suffering from heart disease and bronchitis were far more sensitive to heat stagnation than healthy men ; they were comfortable so long as the temperature readings lay between 8° and 20° C. in spite of the CO_2 rising to 1·5 per cent. Normal individuals became uncomfortable when the temperature rose to 27°–31° C. with a relative humidity of 51–60.

Benedict[3] found that a man could live for many days in health and comfort in his calorimeter chamber so long as the air was kept cool and dry, and have no cognizance of any defect in ventilation when the CO_2 rose to 1–2 per cent.

A wooden chamber of 3 m. cubic capacity was constructed for me at the London Hospital Medical School with large glass observation windows and a man-hole for entering. Inside were electric heaters, coils through which cold water could be circulated, and three electric fans for stirring the air.

Seven or eight students were shut within until the CO_2 reached 3–4 per cent., and the oxygen fell to 17–16 per cent., and the wet-bulb rose to 80°–85° F., and the dry-bulb a degree or two higher. The

[1] *Arch. f. Hyg.*, 1883, **1**, 1.

[2] *Ztschr. f. Hyg. u. Infektionskrankh.*, 1905, **49**, 363, 388, 403, and 433 ; 1907, **57**, 1 and 23 ; 1908, **61**, 148.

[3] *U.S. Dept. Agric.*, Bull. 175, 235.

students went in laughing and chatting, but as the wet-bulb rose became flushed and moist and ceased to talk. One might try in vain to light a cigarette, and borrow matches from another, unaware that the percentage of oxygen had fallen too low for combustion. The breathing was deepened but no headache resulted from short exposure to 3–4 per cent. CO_2. Their discomfort was greatly relieved by putting on the electric fans and whirling the air in the chamber.

They asked for the fans to be put on when they were stopped. The air enmeshed in their clothes was saturated at body temperature; whirling the air of the chamber at 85° wet-bulb, through the clothes and over the skin of the face, relieved the heat stagnation.

The fans lowered the cheek temperature of one student from 34° to 31·5° C. The pulse frequency of seven students was lowered by the fans thus: 92 to 72; 96 to 86; 128 to 84; 94 to 74; 106 to 100; 72 to 52; 92 to 86.

Breathing the air of the chamber through a tube by one outside caused no discomfort.

Stratification of air takes place in artificially heated rooms. Tests made by Bacot with maximum thermometers in a sterilizing chamber showed 37° C. at the floor, 60° C. at the door level, 103° C. at the roof 7 feet above the floor. Attention is directed to this matter as one of importance in connexion with the sterilization of clothes, &c. It is obvious that clothes at the top of the chamber would be sterilized and not those at the bottom. Similarly in the chamber packed with students, the fans whirled up colder air from the floor level. I found there might be several degrees difference between floor and roof level even when the fans were on.

When CO_2 was put into the chamber so as to suddenly raise the percentage to 2, the subjects were unaware of this.

R. A. Rowlands and H. B. Walker carried out a number of work experiments in this chamber, lifting a heavy weight 12 times a minute for five minutes, and observing the effect on the pulse frequency, respiration, and feelings of fatigue when (1) CO_2 was absorbed by means of a caustic soda respirator, (2) allowed to reach a percentage of 4 or 5 and inhaled, the oxygen falling correspondingly, (3) when the wet-bulb was 85°–95°, (4) when cooled down ten degrees by means of the water-cooler.

Relief was effected in every case by cooling, not by altering the chemical purity of the atmosphere.

The following gives an example of the results obtained: [1]

R. A. R. lifted a weight 12 times a minute for series of five minutes. CO_2 absorbed and fans off:

Number of respirations per minute	Total volume breathed per minute	Tidal air	Pulse-rate	Rectal temperature	Temperature of chamber	
					Dry-bulb	Wet-bulb
	Litres	c.c.		Degrees F.	Degrees F.	Degrees F.
10	18	1,800·0	76	99·8	95	87
12	32	2,666·6				
17	25	1,470·5	(Accel. 22)			
26	45	1,730·8				
19	23	1,210·5	98	99·8	99	93

[1] Reported in the *Smithsonian Miscellaneous Collections*, 1913, **60**, 23.

Average volume breathed, 28·6 litres per minute. Average tidal air, 2,219·6 c.c.

On resting, pulse-rate during 1st minute, 98; 2nd minute, 90; 4th minute, 84; 5th minute, 80.

CO_2 let into chamber from bag outside. Fans put on to thoroughly mix atmosphere of chamber; then the fans were put off. Sample taken. Analysis: CO_2 3·52 per cent.; O_2 18·5 per cent.

R. A. R. lifted weight 12 times per minute for series of five minutes:

15	37	1,466·6	82	99·8	93	87
15	38	2,533·3				
17	40	2,352·9	(Accel. 36)			
18	45	2,500				
26	53	2,384·6	118	99·8	99	95

Average volume breathed, 42·6 litres per minute. Average tidal air, 2,247·48 c.c.

On resting, pulse-rate during 1st minute, 118; 2nd minute, 108; 3rd minute, 104; 4th minute, 96; 6th minute, 96.

Water-cooler put on for half an hour.

Air of chamber cooled by 11° to 13° F. (wet-bulb). Sample taken. Analysis: CO_2 4·63 per cent.; O_2 17·50 per cent. R. A. R. lifted weight 12 times a minute for series of five minutes:

20	30	1,500	86	99·8	87	84
19	45	2,368·4				
20	35	1,750	(Accel. 22)			
20	60	3,000				
23	50	2,173·9	108	99·8	85	82

Average volume breathed, 44 litres per minute. Average tidal air, 2,584·6 c.c.

On resting, pulse-rate during 1st minute, 108; 2nd minute, 96; 3rd minute, 92; 4th minute, 92; 5th minute, 88.

The New York Commission on Ventilation [1] found no alteration in the hydrogen-ion concentration of the blood in their subjects sitting in a room at 86° F. with 80 per cent. relative humidity, compared with a room at 68° F. and 50 per cent. relative humidity; the rate of respiration, respiratory quotient, oxygen consumption, and creatinine excretion were all undisturbed in their subjects by the change from the cool to the hot room.

The working of the circulatory and heat-regulating mechanism was markedly influenced even by a slight increase in room temperature, e.g. from 68° to 75° with 50 per cent. relative humidity in both cases. This is illustrated by the following average figures obtained from all subjects at end of day:

TABLE IX

Room temperature	Reclining pulse frequency	Difference in pulse-frequency-increase on standing at beginning and end of period	Average systolic pressure	Average Crampton [2] value	Rectal temperature
86°	74	+7	112 mg. Hg	35	37·41° C.
75	—	−3	116	45	36·99
68	66	−7	116	60	36·73

[1] *Science*, 1915, 41, 625.

[2] The Crampton value indicates the vascular tone. 100 per cent. indicates that the pulse-rate on standing increases less than 5 beats, while the systolic blood pressure increases 10 mm. Hg. Zero value indicates that the pulse-rate increases over 40 beats, and the systolic pressure drops more than 8 mm. Hg.

The appetite for lunch was less in an atmosphere which was allowed to become chemically impure than in one kept pure by ventilation but at the same temperature. A fan was used to stir the chemically impure atmosphere so as to try and keep the conditions in respect to movement of the air the same, but no measure of cooling power was made. It may be surmised that ventilation increased this, and was more efficient in carrying away body heat than the fan, and that the greater appetite was caused by greater cooling power and not, as the Commission suggest, by the purer air.

This lessening of appetite is in contradiction with the statement made by the Commission that no difference was made by a hot moist atmosphere to the oxygen consumption of the subjects.

The experiments detailed in Part I, p. 86 (*Med. Res. Com., Spec. Rep. Ser.*, No. 32) of this report show the powerful effect of cool, open-air conditions on metabolism.

Warmth provokes relaxation and sleep, while cool conditions favour brain work. The Commission report that 'children in over-heated schoolrooms and workers in over-heated factories are listless and inactive'.

Examining 215 different workrooms in New York State, 156 (73 per cent.) had a temperature of 79° F. or over, and 63 (29 per cent.) had a temperature of 80° F. or over. All cases were excluded in which the out-door temperature was over 70° F.

Flüge made observation on 29 days in January and February in seven High School rooms in Germany. At the beginning of school the temperature was over 71·5° F. in three class-rooms eight times, in one 10 times, in one 13 times, in one 16 times, in one 18 times. The temperature frequently rose to 75°, and even to 80° F. When the windows were opened it fell to 57° F.

The next table shows the temperatures recorded on the manoeuvring platform in the engine-room of a destroyer; a slight escape of steam made the wet-bulb very high. Fans or compressed-air douches are greatly required in such places:

TABLE X.—*Temperature, Fahrenheit, wet- and dry-bulb, on H.M.S. Lyra, September and October*, 1911

Date	Where taken	Time 4 A.M.		8 A.M.		Noon		4 P.M.		8 P.M.	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
20–9–11	Engine-room	—	—	—	—	—	—	87	98	85	100
	Deck . .	—	—	—	—	—	—	58	60	55	58
21–9–11	Engine-room	84	96	85	97	82	95	78	96	—	—
	Deck . .	59	60	55	56	55	60	60	62	—	—
22–9–11	Engine-room	—	—	—	—	—	—	90	110	—	—
	Deck . .	—	—	—	—	—	—	61	67	—	—
25–9–11	Engine-room	—	—	95	108	90	105	81	97	80	105
	Deck . .	—	—	57	61	62	67	62	65	60	62
26–9–11	Engine-room	—	—	80	95	85	100	85	108	—	—
	Deck . .	—	—	60	63	63	66	64	66	60	61
27–9–11	Engine-room	75	95	85	102	—	—	—	—	—	—
	Deck . .	57	58	60	63	—	—	—	—	—	—

TABLE X (*continued*).

Date	Where taken	Time 4 A.M. Wet	4 A.M. Dry	8 A.M. Wet	8 A.M. Dry	Noon Wet	Noon Dry	4 P.M. Wet	4 P.M. Dry	8 P.M. Wet	8 P.M. Dry
28-9-11	Engine-room	—	—	86	105	85	105	80	100	98	105
	Deck . .	—	—	56	59	56	58	56	62	58	60
29-9-11	Engine-room	—	—	80	95	84	102	95	115	—	—
	Deck . .	—	—	53	55	51	59	55	61	—	—
3-10-11	Engine-room	—	—	85	100	86	102	85	105	—	—
	Deck . .	—	—	50	50	50	50	56	56	—	—
4-10-11	Engine-room	—	—	84	100	85	100	80	96	—	—
	Deck . .	—	—	46	48	56	58	53	53	—	—

The effect of a hot room on working efficiency is dealt with on page 60 in Part I of this report.

The condition of the vascular system and tone of the belly wall of those exposed to hot atmospheres tends to become comparable to that seen in those who suffer from cardio-vascular instability (effort syndrome of Lewis).

In an examination of 10,000 men at the Syracuse recruit camp 616, or 6·16 per cent., had this condition. (Rothschild.)[1]

Flack has shown that unfit men who are classed as cases of nervous and cardio-vascular instability show a relaxed tone of the belly-wall, 'doughy belly'; they generally are costive, flush on effort, and may give a stomach splash, signs of the slackened tone of their smooth muscle. The fit man can blow against a mercurial manometer and raise it on the average 112 mm. Hg, the unfit 74 mm. Hg. The fit man can hold his breath against a pressure of 40 mm. Hg on the average 52 sec. without any notable acceleration of the heart. The unfit 25 sec. and his pulse accelerates greatly, and may secondarily show great slowing (owing to vagal inhibition) signs of instability of the medullary centre, its over-sensitiveness to the asphyxia condition of the blood. In place of a vital capacity of 4,000 c.c. or over, that of an efficient man, the unfit show a vital capacity greatly lessened, e.g. even to 2,400 c.c. The unfit are shallow breathers; their CO_2 alveolar tension as determined by Haldane's method is lowered, for they have not the power to expire forcibly; the oxygenation of the blood is less in them owing to the imperfect expansion of the lungs. In shallow breathing some parts of the lung remain unexpanded, and the blood which passes through them unoxygenated. The fit man set to breathe out against a resistance of 40 mm. Hg can maintain his vital capacity almost undiminished. The unfit man cannot do this. Set to lift his body weight on to a chair (30 inches high) five times in 15 seconds at regular intervals, the fit man's pulse-rate increases by 12 to 24, and on resting returns in 10 to 20 seconds to the normal. The unfit man's pulse increases by over 26, and does not return to normal in 30 seconds. Emotional excitement may accelerate the pulse in the fit and must be checked before carrying out this test. The diastolic pressure is low in the unfit, and the pulse-pressure (difference between systolic and diastolic pressure) great. His pulse-rate and pulse-pressure are considerably increased on rising from the recumbent to the vertical posture.

[1] *N. York M. J.*, 1913, **98**, 916.

The febrile patient, he who is suffering from an acute catarrh or made ill by other infection, or by wound, or by irritant gas-poisoning of the lung, and the war pilot exhausted by perils of flying and fighting, by want of oxygen, or by the shock of a crash, alike fail in all these tests for fitness.

I cite as an example this report [1] on a moderately severe case of paratyphoid fever with considerable abdominal distension. From the thirty-third day onward the pulse and respiration appeared normal with the patient recumbent in bed. On leaving bed for the first time on the fortieth day, and standing up, the patient felt faint and giddy, and the pulse went up to 150. The putting on of a tight abdominal bandage made the patient more comfortable, and lowered the pulse to 120. Under the influence of gravity the blood-supply was depleted from heart and brain into the great abdominal veins owing to the lack of tone of the belly-wall. Not only active infection with many varieties of organisms, but even vaccination with dead bacilli, may give rise to instability of the vaso-motor system, signalized by tremor, sweating, clammy hands, dermographia, and tachycardia, the pulse being rapid during sleep and on standing up, the subject not being able to hold the breath long. The heart-muscle may be poisoned and weakened in addition to the loss of vascular tone.

The loss of vascular tone is intensified by exposure to sun or hot atmospheres. Those infected and febrile are most liable to go under in the tropics from heat-stroke. In the open-air ward both the febrile and the wounded do best.

With a wind of 0·5 metres per sec. a temperature of 20·3° C. gives a dry kata cooling power of 10. Suppose the air temperature were 10° C., the same cooling power would be obtained if the temperature of the surface of the kata were not 36·5° but 26·2° C. So, too, the skin is cooled by vaso-constriction on exposure to cool wind, so that the cooling of the body may be kept constant.

The unfit conditions tend to become manifest in the sedentary workers who are fatigued by long hours, and whose bodies are relaxed by work in over-warm atmospheres, and who take little or no exercise in the open air. Passed otherwise as A1 men, they fail in the test for fitness and cannot be accepted as air-pilots.

The treatment found of avail for those convalescing and suffering from unfitness is open air and exercise, never carried to over-fatigue, with sea-bathing in addition if possible.

The absorption of toxic products from disturbed function of the bowel or from some infection intensifies the conditions of unfitness induced by nervous exhaustion and a hot close atmosphere. Long hours of sedentary work in close atmospheres tend to produce unfitness from all these causes.

Women are especially prone to the faintness induced by work in close atmospheres. It was reported by Vernon [2] that at a war factory the number of workers treated for faintness was six times greater for women than for men during day shift, and four times greater during night shift. The number of cases treated with sal-volatile was 23 times greater for women than for men in the early war period when

[1] H. F. Marris, *Lancet*, May 11, 1918.

[2] See *Final Report of Health of Munition Workers Committee.*

the hours were too long, subsequently only two or three times more numerous.

Women are brought up more confined, less exercised. They have to meet the periodic vascular congestion of their sexual organ, and the adequate development of their abdominal muscles is neglected. They suffer, then, more from fatigue from standing, and circulatory failure in the erect posture. The condition of the circulation in those exhausted by exposure to heat may be compared with that produced by shock.

After some minutes' exposure to a hot bath (102–10° F.) with all but the face immersed, the rectal temperature may reach 103° F., the pulse 150 per min., the systolic blood-pressure sink to 80 mm. Hg, the respiration become rapid and shallow, the rate may rise to 30 per min., pulmonary ventilation to 50 litres per min. There may be feelings of oppression and palpitation.

The cutaneous capillaries dilate and the blood is pooled within them, and on rising into the vertical position the subject may turn faint owing to the blood sinking under the influence of gravity into the dilated vessels of the lower part of the body. The symptoms, with the exception of the rectal temperature, are immediately relieved by a cold douche, which constricts the cutaneous vessels and cools the skin.

The condition of shock is produced by several causes; it may arise from intense nervous excitation, e.g. emotional shock, from absorption into the blood of toxic products, probably of protein origin, arising from traumatized tissues, from absorption of bacterial toxins and certain other poisons, from severe haemorrhage, from want of oxygen in the air breathed, from exposure to excessive heat.

When traumatic shock is well established no relief can be given by any measures taken to confine the capillaries. The blood is not only pooled within them, but fluid has transuded out.

Crile [1] upheld the view that traumatic shock is due to exhaustion of brain cells by intense sensory excitation, and that such could be prevented by local anaesthesia which blocked the sensory nerves. By suitable anastomosis of blood-vessels, he cross-circulated two dogs so that the blood of each freely intermingled; only the animal submitted to traumatism showed the characteristic changes in the brain cells; these, then, were produced not by products of traumatism in the blood, but by sensory excitation. Severe traumatism of the brain itself did not produce the changes in the brain cells.

No shock was produced in 'spinal animals' by traumatism of the body below the point of section of the spinal cord. Similar changes in the brain cells were produced by fear, exhaustion through excessive activity, prevention of sleep, and great loss of blood.

The Committee upon Shock appointed by the Medical Research Committee has brought forward evidence [2] which favours the view that shock can be brought about by absorption of a substance or substances from killed and dying tissues; amputation of a severely damaged limb may relieve shock by putting an end to such absorption. So, too, in the case of extensive burns of the skin, shock is regarded as resulting from absorption of products of protein decomposition. Local trauma—

[1] *Lancet*, July 5, 1913.

[2] *Medical Research Committee, Special Report Series*, No. 25.

a burn or the wheal of a cane—causes capillary flushing and oedema, provoked by the damaged tissue products.

If the nerves of a limb be divided and the blood-vessels clamped, and then the muscles be submitted to traumatism, no symptoms of shock ensue until the vessels are opened. (Bayliss.)

On the other hand, there is the statement that no shock can be produced in 'spinal' animals by traumatism of the body below the point of section of the spinal nerves.

Not only extensive burns or laceration of tissues, but lack of oxygen in the air breathed, or arising from deficient blood-supply (haemorrhage), may, it is thought, initiate tissue changes which lead to the appearance of the substances which produce shock.

Most noteworthy is the fact that an etherized cat can be put into the condition of shock by an injection of histamine, but not the normal cat, or one anaesthetized with nitrous oxide and oxygen. (Dale.) On the one hand there is the cumulative effect of two poisons, and on the other the protective effect of oxygen.

In the state of shock produced by injection of histamine in the etherized cat the whole of the potentially available capillary channels become patent; the blood percolates into the network of channels as into a sponge. The morbid permeability of the capillary wall, or increased osmotic pressure of tissues, lessens the blood-volume; 50 to 60 per cent. of the plasma may pass out, i.e. in the cat 40 per cent. of the blood-volume. A constriction of the arterioles holds up the arterial pressure, but the venae cavae in the thorax are flaccid, half empty, the portal vein flat; the filling of the heart in diastole wanes. The liver and spleen are moderately pale, but the bowels show a diffuse dusky congestion. (Dale, Laidlaw, and Richards.)

We must not regard the walls of the blood-vessels as alone supporting the blood-pressure as in an artificial schema, for the whole of the water-swollen living substance confined within elastic and muscular membranes, the extension of which is limited by white connective tissue, helps in their support. The force of imbibition of the muscular fibres confined by sarcolemma, of gland cells confined by membrane propria, of tissue cells in general confined by connective tissue arranged everywhere for that purpose, supported by aponeuroses, capsules of organs and skin, this force of imbibition of the living cells supports the blood-vessels, arteries, capillaries, and veins.

The equilibrium of chemico-physical activities of the cell protoplasm, which maintains the water content and swelling, is the chief regulator and maintainer of the capillary circulation.

The arteries, too, require the support of the tissues. Experiment on the measurement of blood-pressure by means of the sphygmomanometer show a far lower pressure will prevent the passage of the pulse-wave if applied to a naked artery than if applied to the artery *in situ* in a limb. The return of the venous blood depends on the changes of posture and muscular movements, the contraction of skeletal muscles, the pumping action of respiration, the contraction of involuntary muscle of the bowels, &c. The circulation could not possibly persist in blood-vessels isolated from the tissues; it fails when the tissues become flaccid.[1]

[1] Leonard Hill, *Lancet*, 1920, i. 359.

Using two Leclanché cells and zinc electrodes covered with chamois leather soaked in normal saline, Waller[1] finds the electrical resistance between the back and palm of the hand is generally between 10,000 and 40,000 ohms. In people of nervous temperament the resistance is lowered by emotional changes of consciousness, probably an index of the change brought about in the imbibition forces of the cell by nervous shock. The concentration of ions on the cellular membranes and imbibition of fluid by the cells are changed so that a generalized passage of fluid into extra-cellular spaces and flaccidity results.

One of the most important functions of cooling winds is to excite and keep up the tone and hardness of the body by day, while by night the body is relaxed during sleep taken in recumbency in a warm place.

The blood-capillaries afford vast potential channels, but are mostly contracted down and empty in the tonically contracted vigorous body. The body may be regarded, for the purpose of illustration, as a wet sponge enclosed in a rubber bag and confined by the elasticity of the rubber so that its pores are narrowed, and many even empty of water. Suppose the rubber bag suddenly becomes distensile and enlarges, the pores of the sponge all open out and the water is determined by hyperstatic congestion (by gravity) to the lower parts. So is it with the body generally relaxed by heat, wound, or emotional shock. But the relaxation due to heat can be abolished by cold—the neuro-muscular mechanism is efficient. In shock, on the other hand, the mechanism is out of action. it is most difficult to arouse the tone, and so confine the capillaries and restore the circulation.

Recent work of Krogh shows that most of the capillaries in the resting muscles are in a state of contraction and closed to the passage of blood. In active muscles a large number open up. He concludes that clinical hyperaemia and anaemia are due mainly to changes in the calibre of capillaries and in the number open.[2]

The contractility of the capillaries of the skin has been demonstrated by T. Lewis and his co-workers. Capillaries are dilated by excitation of sensory nerve fibres—axon reflexes, or by metabolic substances produced in active cells, and in my opinion the dilatation is due rather to change in the imbibition of the tissue cells than to active alteration of the capillary wall.

For life to continue, the hydrogen-ion concentration of the blood has to be kept within very narrow limits. The view has been put forward that shock is due to 'acidosis' produced by a reduction of the alkaline reserve of the blood through rapid shallow breathing and increased pulmonary ventilation, and reduction thereby of the carbon dioxide concentration in the blood.

If the arterial pressure be lowered below 70 mm. Hg by obstructing the filling of the heart by means of a cannula tied into the pericardium connected with a column of salt solution, and the pressure be kept at this low level for over an hour, the alkali reserve of the blood is lowered through oxygen-want increasing the pulmonary ventilation. (Cannon.)

Now when by increased ventilation carbon dioxide is removed from sodium bicarbonate in the blood, sodium carbonate is formed, and this is removed by the tissues from the blood so that the neutral

[1] *Proc. Roy. Soc.*, 1920, **B. 91**, 17. 32. [2] *J. Physiol.*, 1919, **52**, 457.

reaction of the blood may be preserved; thus the alkaline reserve is diminished and the serum has a lessened power of combining with carbon dioxide (as shown by van Slyke's method).

The condition is clearly not one of acidosis but of alkalosis, as was first clearly pointed out by Benjamin Moore.[1] By removal of carbon dioxide the alkali in the body must be increased unless removed by the kidneys, or neutralized by formation of acid, or balanced by diminished formation of ammonia in the liver. An addition of acid to the blood appears to be immediately compensated for by a washing out of carbon dioxide by increased pulmonary ventilation.

'To maintain a rise of H-ion concentration which causes persistent vascular dilatation, an infusion of acid must be made altogether outside physiological limits, and such as would cause death, not slowly from shock, but rapidly from acute heart failure.'

On the other hand the body appears to hold on to its alkaline salts, and time is required to adjust the alkalosis produced by increased pulmonary ventilation occasioned by want of oxygen. The addition of bicarbonate of soda to salt solutions used for transfusion has therefore been wrongly advocated in the treatment of shock.

The evidence put forward by the Special Investigation Committee on Surgical Shock[2] proves that 'simple "acidosis" in the sense of a reduction of the alkali reserve of the blood, even though it be severe and prolonged, does not cause shock or, indeed, any perceptible impairment of the circulation or other vital functions in an otherwise normal animal at rest.'

Lewis, Barcroft and others correlate breathlessness in cases of cardio-vascular debility with a reduction of the alkali reserve of the blood. Such is the case also in 'gassed' soldiers and animals.

In polypnoea excited by exposure to heat the respiration becomes rapid and shallow, to promote evaporation from the respiratory membrane. A dog thus cools itself, panting even up to 300–400 times per minute. The polypnoea is of central origin, not influenced by division of the vagi. On closing the trachea it continues $1\frac{1}{2}$ to 2 minutes—the blood being super-oxygenated—asphyxial respiration then ensues. The question arises as to whether polypnoea, by causing an alkalosis of the blood, produces heat exhaustion. Evidence shows that carbon dioxide is washed out by the polypnoea excited by heat.

In the tropics and in hot weather compared with cold, a lower partial pressure of CO_2 is obtained in the alveolar air[3] by Haldane's method, i.e. expiring forcibly through a tube and taking a sample for analysis from the tube. The alveolar concentration of CO_2 is also lowered in a hot bath through polypnoea.[4]

It must be borne in mind that the question has been raised as to whether the partial pressure of CO_2 is really lowered in the alveoli of the lung, or whether the Haldane method fails to give the true reading. If the tone of the belly-wall is lowered, and the subject is therefore unable to expire so deeply, he will have an apparent reduced vital capacity and lessened alveolar concentration of CO_2. Flack finds an

[1] Later accepted by J. Haldane and Y. Henderson.

[2] *Medical Research Committee, Special Report Series*, No. 25, 1919, 245.

[3] Chapman and Wardlow. *Med. J. Australia*, 1916, 387; Boycott and Haldane, *J. Physiol.*, 1908, **37**, 355; W. A. Osborne and others, *Proc. Roy. Soc.*, 1920, **B. 91**, 111.

[4] Flack and L. Hill, *Proc. Physiol. Soc.*, Mar. 27, *J. Physiol.*, 1909, **38**.

unfit air-pilot (crashed or war-worn) may have his vital capacity lowered from over 4,000 to 2,300 c.c.

In the case of altitude sickness the reaction to oxygen-want comes on in fit men at about 18,000 feet, when the oxygen partial pressure is reduced to about one-half what it is at sea-level. The lips appear cyanotic, the pulse-rate increases about 30 per cent., the pulse-pressure about 36 per cent., mainly by fall of diastolic pressure. The respiration becomes slower and deeper, the ventilation being increased about 50 per cent.[1] The visual reaction time is lengthened 25 per cent., and the mentality altered, fixity of ideas is a common symptom, powers of calculation and observation diminish. Carbon dioxide is washed out by the increased pulmonary ventilation and the alkaline reserve of the blood diminished. The alkalosis set up occasions excretion of alkaline urine and diminished formation of ammonia.[2]

Moore[3] points out that in shock, while the portion of the blood which is pooled in the relaxed capillaries of the flaccid body may be strongly charged with CO_2, the portion passing through the lungs is over-denuded of CO_2, and, reaching the heart and brain, may produce a degree of alkalosis there which disturbs the vital reactions.

The tension of CO_2 in the blood going to the brain may thus be reduced by increased pulmonary respiration arising from (1) forced breathing, (2) exposure to attenuated air at high altitudes, or greatly reduced partial pressure of oxygen at sea-level, (3) carbon monoxide poisoning, (4) severe haemorrhage, (5) the state of shock, (6) exposure to hot baths or hot moist atmospheres which pool the blood in the capillaries. Forced breathing, exposure to heat, haemorrhage, shock, not only washes CO_2 out but, impeding the circulation, causes oxygen-want.

All these conditions, then, produce oxygen-want. They are all benefited by oxygen inhalation—forced breathing, for example, cannot be kept up for long when breathing air, but can be kept up for longer, with consequent greater reduction of CO_2 in the alveolar air, on breathing oxygen. (L. Hill and M. Flack.)

All these conditions appear to be benefited also by breathing air containing 1–2 per cent. of carbon dioxide. The stimulus thus given to the respiratory centre by the increased hydrogen-ion concentration results in less shallow breathing and better oxygen supply.

It was observed by Mosso, in the hut on the top of Monte Rosa, that strict attention to a skilled operation might reduce the respiratory activity to such an extent that want of oxygen ensued in the brain, as evidenced by dizziness. This is an observation of great interest, as it points to the fixation of the thorax and limitation of breathing during the performance of skilled hand operations.

Muscular exercise, by increasing the partial pressure of carbon dioxide, naturally secures deep breathing and thorough oxygenation of all parts of the body. The importance of this cannot be over-stated. After very hard exercise there may be a fall in the alkaline reserve of the blood lasting several days.[4] This is attributed to the formation of lactic acid in the muscles owing to the oxygen supply not meeting the

[1] *Rep. Air Medical Investig. Comm. M.R.C.*, No. 5, 1918.
[2] Haldane, Kellas, and Kellaway, *J. Physiol.*, 1919, **53**, 181.
[3] *Brit. M. J.*, Sept. 7, 1918.
[4] Lusk, *Science of Nutrition*, 362.

demand during the exercise, hence the feelings of stiffness and fatigue. The breathing of oxygen during or before and after severe athletic exercises prevents the formation of lactic acid and such after-effects. (L. Hill and M. Flack.)

The hyperpnoea produced by severe work, through shortage of oxygen and production of lactic acid, at first washes out preformed CO_2 from the body, giving an abnormally high respiratory quotient. At the end of the work there is another sharp rise of respiratory quotient due to washing out of CO_2 dammed back in body during the work. After this the respiratory quotient may go back to its old resting level, or to a considerably lower level resulting from washing out of CO_2 or depletion of carbohydrate by work.[1]

Sweating may have an important effect on cellular metabolism, just as bleeding, purgation, and diuresis must have, for diminution in the volume of cells, through loss of water, increases the internal concentration of substances to which the cell membrane is impermeable. By mass action reactions of which these substances are components may be accelerated. Increased absorption of water may retard such reactions.[2] We know that the equilibrium of the colloidal cell substances, with a solution of certain salts of strength equal to the blood, has to be maintained for the phenomena of life to continue.

When distilled water is drunk to make good loss of weight produced by sweating, it is rapidly lost through the skin and kidneys. Weight can only be regained by taking enough salts with the water or in food to replace those lost in the sweat. (Cohnheim.)

After drinking much water urine is passed nearly equal to pure water: as much may be passed in an hour as normally in 24 hours. The blood shows a very slight decrease in the percentage of salts. After drinking a dilute solution of salt there occurs the same increased secretion of urine, but an easily measurable dilution of blood, the salt being slightly in excess. Both water and salt pass out, but at first very little of the salt. (Haldane and Priestley.)

After loss of colloid serum protein out of the blood in wound shock or by severe haemorrhage, isotonic salt solution will not restore the circulation, but saline with 5 per cent. gum arabic—a colloid—will do so. (Bayliss.) After loss of water and crystalloids through diarrhoea (cholera), saline alone will restore the circulation. Gum saline is contra-indicated. (L. Rogers.)[3] According to Bayliss the chief function of the serum protein is to maintain the osmotic pressure viscosity and the immunizing powers of the blood; it has no nutritive function.

The effect of heat stagnation on the internal secretions must be considered, in connexion with the view put forward by W. Cramer,[4] and already referred to, that the thyroid and adrenal glands functioning together form an apparatus for the heat regulation of the body, a humoral mechanism supplementing the nervous one.

Increased secretion of the thyroid provokes increased secretion of adrenin, and this in its turn leads to the mobilization of glycogen into sugar and increased heat production. In the absence of carbohydrate,

[1] Campbell, Douglas, and Hobson, *Proc. Roy. Soc.*, B. 1919, Dec. 11, *Phil. Tr.* **B. 210**, 1.
[2] Cf. Bayliss, *loc. cit.*, 163.
[3] *J. Physiol.*, 1919, **53**, 162.
[4] *Sc. Rep. Imp. Cancer Res. Fund*, 1919, **6**, 1.

protein and perhaps fat may be used up in the increased glycogenic function.

The secretion of adrenin, if active enough, may contract the cutaneous arterioles and lessen heat loss. Cramer finds that moderate exposure to cold keeps the adrenal glands loaded with secretion. Excessive chilling depletes the store, but to effect this mice have to be wetted and exposed in a chamber at 5° C. Exhaustion of the adrenals is brought about by exposure of mice not only to excessive chilling but to heat, e.g. 40–45° C.

The thyroid under these conditions also shows changes such as extreme congestion and vacuolization of cells. Severe bacterial infections exhaust the adrenals, so too haemorrhage, but not operative shock. Excessive chilling or over-heating, by helping to exhaust the adrenals, contribute to the fatal outcome of infections.

The infected, then, not the healthy, require protection from cold. The evidence drawn from the study of war nephritis [1] may here be cited, which shows that exposure to cold is not a cause, but that *after infection* exposure to very severe climatic conditions may compel patients to report sick at an earlier period, the resistance to cold being lowered by infection.

Pyrexia in infectious disease signifies hyper-adrenal action, a subnormal temperature exhaustion of the adrenals.

In this connexion it is noteworthy that hyper-action of the thyro-adrenal mechanism, owing to excessive emotion, has been put forward as a cause of war neurasthenia. In several early cases exophthalmos and other symptoms of Graves's disease were noted.[2] Cannon found that emotions of fear and anger in animals caused hyper-adrenal action. This excites vaso-constriction of cutaneous blood-vessels and dilatation of the vessels of the muscles.[3] Exhaustive hyper-action of the thyroid and adrenal, resulting from the emotional life and worry of modern society, contribute to unfitness, and is to be counteracted by the quiet and coolness of open-air life.

[1] H. MacLean, *Medical Research Committee, Special Report Series*, No. 43, 1919, 57.
[2] W. Johnson, *Brit. M. J.*, Mar. 22, 1919.
[3] Cf. Swale Vincent, *Endocrinology*, 1919, 3, 121.

CHAPTER V

Health and Environment[1].

Ellsworthy Huntington[2] from an examination of the death-rate in U.S.A. and Europe concludes that health is best when the average temperature is about 64° F., that is about 70° at noon and 55° at night, —that is when windows are open and artificial heat is not required. He finds evidence that health is the most potent cause of national prosperity, and that health depends primarily on weather.

In the middle of the eighteenth century the bulk of the nation must have consisted of small communities of localized and specialized types, largely engaged in agricultural pursuits. The census of 1811 showed that 35 per cent. of the population of Great Britain was so engaged. The increase of industrialism in the nineteenth century was such that in 1913 only 8·8 per cent. of the population was engaged in agriculture, forestry, and fishing, as compared with 69 per cent. in Hungary, 42·7 per cent. in France, 35·2 per cent. in Germany.. A very large proportion of the nation has, then, been called upon to adapt itself to conditions contrary to those under which the ancestral stocks evolved in rural life.[3]

During the last 50 years there has been, as the 'expectation of life' tables show, a large extension of life in the early, but not in the later, years. At babyhood the mean after-life time has gone up eleven years for males, and for females fourteen years since 1838–54. At 30–35 years for males the rise of extension of life has dropped to two, for females to four. The mode of modern life tells more against the male.

Table XI.—*Mean after-life time at various ages* ('*Expectation of Life*') *for four English life tables* (in years, upper figures males, lower figures females).

	1838–54	1871–80	1881–90	1891–1900	1901–10	1910–12
0.	39.91	41.35	43.66	44.13	48.53	51.50
	41.55	44.62	49.15	47.77	52.38	55.35
1.	46.65	48.05	50.97	52.22	55.68	57.51
	47.61	50.14	53.24	54.53	58.31	60.31
2.	48.53	50.14	53.04	54.12	57.00	58.53
	49.40	52.22	55.18	56.34	59.58	61.28
3.	49.61	50.86	53.32	54.26	56.92	58.32
	50.20	52.99	55.46	56.49	59.49	61.09

[1] I am indebted to Dr. M. Greenwood for a critical revision of this chapter.
[2] *World Power and Evolution*, New Haven, 1919.
[3] J. S. Mackintosh, *Brit. M. J.*, Aug. 2, 1913.

TABLE XI (*continued*).

	1838–54	1871–80	1881–90	1891–1900	1901–10	1910–12
4.	49·81	51·01	53·15	53·98	56·49	57·80
	50·43	53·20	55·31	56·25	59·09	60·58
5.	49·71	50·87	52·75	53·50	55·90	57·14
	50·33	53·08	54·92	55·79	58·53	59·94
6.	49·39	50·38	52·19	52·88	55·21	56·42
	50·00	52·86	54·35	56·18	57·85	59·22
7.	48·92	49·77	51·50	52·16	54·42	55·64
	49·53	51·94	53·65	54·47	57·09	58·46
8.	48·37	49·10	50·73	51·36	53·59	54·82
	48·98	51·26	52·85	53·68	56·27	57·64
9.	47·74	48·37	49·88	50·51	52·71	53·96
	48·35	50·83	52·00	52·54	55·41	56·79
10.	47·05	47·60	49·00	49·63	51·81	53·08
	47·67	49·76	51·10	51·97	54·53	55·91
15.	43·18	43·41	44·47	45·21	47·31	48·57
	43·90	45·63	46·55	47·61	50·08	51·44
20.	39·48	39·40	40·27	41·02	43·01	44·21
	40·29	41·66	42·42	43·44	45·77	47·10
25.	36·12	35·68	36·28	37·01	38·86	40·00
	37·04	37·98	38·50	39·37	41·54	42·80
30.	32·76	32·10	32·52	33·07	30·76	35·81
	33·81	34·41	34·76	35·39	37·36	38·54
35.	29·40	28·64	28·91	29·24	30·79	31·71
	30·59	30·90	31·16	31·52	33·31	34·37
40.	26·06	25·30	25·42	25·64	26·96	27·74
	27·34	27·46	27·60	27·52	29·37	30·30
45.	22·76	22·07	22·06	22·20	23·27	23·92
	24·06	24·06	24·05	24·20	25·53	26·34
50.	19·54	18·93	18·52	18·90	19·76	20·29
	20·75	20·68	20·56	20·64	21·81	22·51
55.	16·45	15·95	15·74	15·79	16·48	16·89
	17·43	17·33	17·23	17·24	18·27	18·87
60.	13·53	13·14	12·88	12·93	13·49	13·78
	14·34	14·24	14·10	14·10	15·01	15·48
65.	10·82	10·55	10·31	10·34	10·80	10·99
	11·51	11·42	11·26	11·27	11·99	12·36
70.	8·45	8·27	8·04	8·05	8·39	8·53
	9·02	9·95	8·77	8·78	9·25	9·58
75.	6·49	6·34	6·10	6·15	6·41	6·49
	6·93	6·87	6·68	6·70	7·10	7·29
80.	4·93	4·79	4·52	4·62	4·86	4·90
	5·26	5·20	5·00	5·05	5·36	5·49

A fact of significance in our vital statistics is the way in which rural districts maintain their relative advantage over town districts, despite the absolute decline of mortality in all districts. For all England and Wales the standardized death-rates of males in 1901–10 was only 85 per cent. of what it had been in the previous decennium, the female death-rate only 83·3 per cent. of what was obtained ten years earlier. But in 1891–1900 urban male mortality was 137·9 per cent. of rural male mortality (standardized rates), and in 1901–10 it was still 134 per cent. of rural mortality. The figures for women are nearly the same, 134·7 per cent. and 132·1 per cent. M. Greenwood tells me

that even this very slight approximation may be illusory and due to an urbanization of the nominally rural counties. A comparison made by Hilda Woods of particular rural with particular urban areas, deemed to be typical, revealed absolutely no change in the 30 years from 1881. In each successive decennium the country dwellers maintained an advantage of 35 per cent. in males and 30 to 33 per cent. in females. When we recollect that the pressure of public opinion and the efficiency of sanitary administration have for more than a generation been greater in the towns, with their whole-time medical officers of health and skilled staffs, we have to recognize that some other factor than what is comprised in the technique of public sanitation plays a great part.

Precisely the same conclusion follows from a different line of statistical research. Sixty years ago, Farr inferred that the death-rate varied approximately as the tenth root of the density of population, and Brownlee has recently shown that the relation is still valid in England of our own time with its improved water supplies, scavenging of towns, and public cleanliness. Why is it that so much effort has failed to lessen the distance between the town dweller and the countryman?

Let us first glance at some facts relative to industrial employment. Of 2,425,184 men graded[1] in 1917 for the army 36 per cent. were placed in grade I, 31–2 per cent. in grade III (C 3 men), 10 per cent. in grade IV (unfit for any military service).

Over 70 per cent. of coal miners, men who do strenuous work in well-ventilated atmospheres, who are well fed and have holidays, were graded I, against 20 per cent. in the case of a sample of cotton operatives who work in moist, over-warm atmospheres. The miners' houses in Ayrshire are the worst both in structure and cubic space, but the other good conditions far more than counterbalance bad housing. The men of Sheffield, mostly iron and steel workers, were graded considerably higher than the men of Leeds, mostly clothiers (many dirty aliens). Of 261,137 boys medically examined, aged 17–18, 65 per cent. were graded I. At Birmingham, Nov. 1917–April 1918, between the ages of 18 to 41, grade I fell from 60 to 4·5 per cent., while grade III rose from 6 to 63 per cent. During May 1–Oct. 31, 1918, the grading was higher, grade I falling from 68 to 19 per cent., and grade III rising from 13 to 61 per cent.

Owing to the war much attention was devoted to the industrial employment of women, and the subject is especially important since, for the first time, large numbers of women were introduced to factory life who had previously been exempt from it. In this country the death-rate of women from tuberculosis rose considerably. Taking the standardized rate of 1912–14 as 100, the rate of 1915 was 106, that of 1917, 113. This cannot have been entirely due to industrial employment, since there was an appreciable increase at ages 5–15, but the bulk of the change was between the ages of 15 and 45, i.e. amongst women of employable age. Further, as Greenwood has pointed out, a redistribution of the female population of the kind observed during the war would, had the differential rates noticed in foreign countries

[1] *Rep. on Phys. Exam. of Men of Military Age.* Cmd. 504.

applied here too, have produced just such an increase as we actually experienced.

We have no English data of female industrial sickness, but many have been published abroad.

Prinzing[1] cites the Frankfort experience of 1896, in which year the incidence of illness per 100 domestic servants was 58·6 cases per annum without, and 29·6 with, industrial incapacity. This does not necessarily mean 58·6 and 29·6 different persons, for the same person may be ill more than once in a year, but it *does* mean that for every 100 years of life lived by domestic servants there will be 58·6 cases of illness not grave enough to incapacitate the victim from service and 29·6 serious enough to lay her up. Yet the domestic servant is a favoured person in respect of illness compared with her sister in the factory. According to the experience of the Leipzig State Insurance Bureau, the annual sickness rate of domestic servants between the ages of 15 and 35 was only 58 per cent. of the average of insured women of that age (see Greenwood); bookbinders—a great Leipzig industry—had twice as much sickness and more than four times as much tuberculosis. The annual case rate of tuberculosis was 2·2 per 1,000 for domestic servants, 9·5 for bookbinders, and as much as 14·7 for women employed in tobacco factories.

Our wide experience of industrial mortality amongst men shows that certain industries suffer a very heavy incidence of tuberculosis, without considering those trades, such as the silica industries and some forms of mining, which entail specific dangers.

Since tuberculosis is the principal cause of death between the ages of 15 and 40 it will be well to discuss the matter further.

Annual Mortality per 1,000 *from Pulmonary Tuberculosis and Phthisis, England and Wales, Age Standardized* (*Supplement to 75th Annual Report of R.-G.*).

Period	Males	Females	Males, taking rate 1851–60 as 100	Females, taking rate 1851–60 as 100
1851–60	2·69	2·85	100	100
1861–70	2·61	2·58	97	91
1871–80	2·36	2·12	88	74
1881–90	1·97	1·67	73	59
1891–1900	1·63	1·23	61	43
1901–10	1·36	0·95	51	33

The decimal rates since 1851–60 are shown in the Table. The rate for males in 1901–10 was half, and for women a third of what it had been 50 years before, but it has not declined so rapidly since 1890 (i.e. in the epoch of anti-tuberculosis campaigns) as it did between 1870 and 1890.

Pearson, who emphasized these facts, is sceptical as to the value of sanatoria, and attributes importance to the selective multiplication of the unfit due, *inter alia*, to birth control by the healthier and more self-respecting citizens. Thus sanitary improvements, especially in the towns, are partly neutralized by the growth of an inferior population which may ultimately lead us to the ruin which attended the growth of the Roman mob in imperial times.

[1] *Handbuch d. mediz. Statistik*, 1906, p. 138.

I recognize the importance of this factor, but suggest that others not less important have been at work. In the last decades, the rush and excitement of modern city life has increased, together with the confinement of workers to sedentary and fatiguing occupations in close atmospheres, and during a period within which housing has improved but little.

That the heavy incidence of phthisis upon industrially employed males[1] can hardly be a mere effect of inherited or innate weakness is suggested by Fig. 26, which shows that with urban males, rural males, urban females, and rural females, the phthisis curves go together for all classes up to the age of about 15–20. Up to this age for all classes the phthisis mortality is about the same. Then we come to the age when they go into occupations, the working age; we find the urban male phthisis curve rises in a striking manner, while the rural male, rural female, and urban female run together, the rural male curve being distinctly below in the later age periods. In Belfast, where female labour is chiefly employed in the spinning and weaving sheds, i.e. in warm, moist atmospheres, the phthisis curve for females aged 10–40 over-tops that for males (Fig. 27). Discount need not be made for the diminution of males through voluntary enlistment in the army, because the tubercular soldiers came back to Belfast to die.[2]

Greenwood attempted to sort out industrial groups especially subject to tuberculosis by considering the correlation between the death-rate from all other causes—used as some measure of physique and industrial status—and from tuberculosis. He examined three English decennial records and one Dutch series. In all these, book-binders, printers, tailors, and cabinet-makers were found to suffer excessively from tuberculosis much more than was to be expected from their death-rates due to other diseases. In order to allow for selection, he related the death-rate from phthisis at ages 35–45, not only with the death-rate from other causes in the same decennium but also with the death-rate of persons employed in the same industries and aged 25–35, ten years earlier. He found that the conclusions were unaffected, and that the partial correlation between the phthisis mortality of one decennium and the mortality of persons ten years younger in the previous decennium was positive. While he fully recognized that, owing to changing of trades, this attempt to measure the selective effect of previous mortality was imperfect, he suggested that it was difficult to believe physical selection alone could account for the incidence upon the occupations mentioned (he excluded from his data all occupations liable to specific hazard of lung disease).

An investigation in the Birmingham area disclosed evidence (*a*) of a relatively greater incidence of phthisis upon the employees in hygienically unsuitable factories, (*b*) of a serious condition of over-crowding in industrial dwellings, but (*c*) no evidence of any specific trade habit amongst munition workers specially apt to favour the conveyance of phthisis from person to person.

[1] Greenhow, Matthew Hay, Barwise, and others. Cf. Collis, Milroy Lectures, *Public Health*, 1915, **28**, 252, and 292.

[2] Among cotton operatives (Fall River, U.S.A.) ages 15–45, the male death-rate from tuberculosis is 51 per cent. above non-operatives, the female 142 per cent. and in spinning rooms 207 per cent.! *U.S. Bur. Lab. Stat., Bull. 251.*

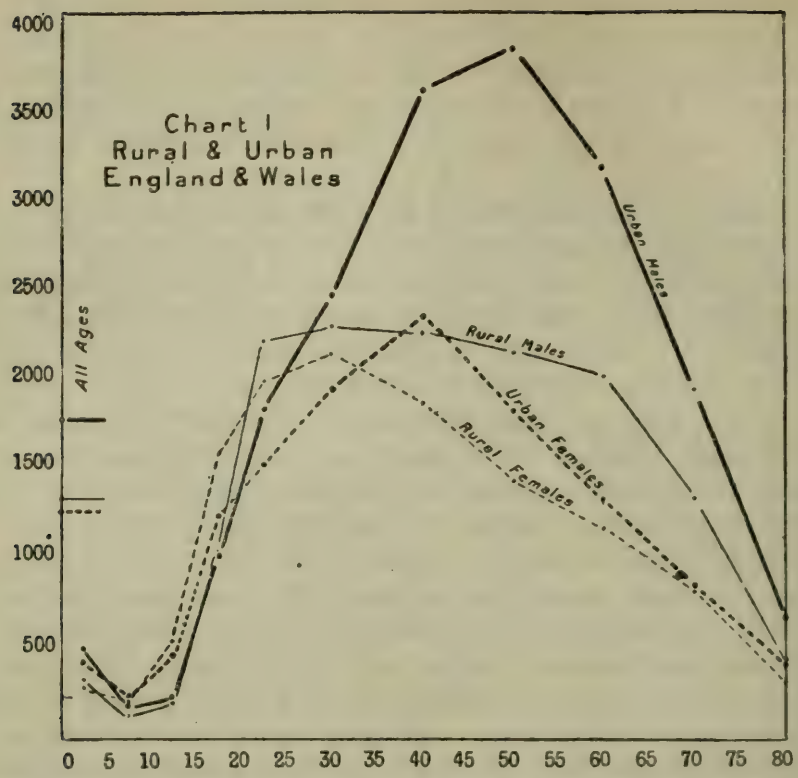


FIG. 26.—Phthisis death-rates per million at ages shown on the base line. B. Moore.

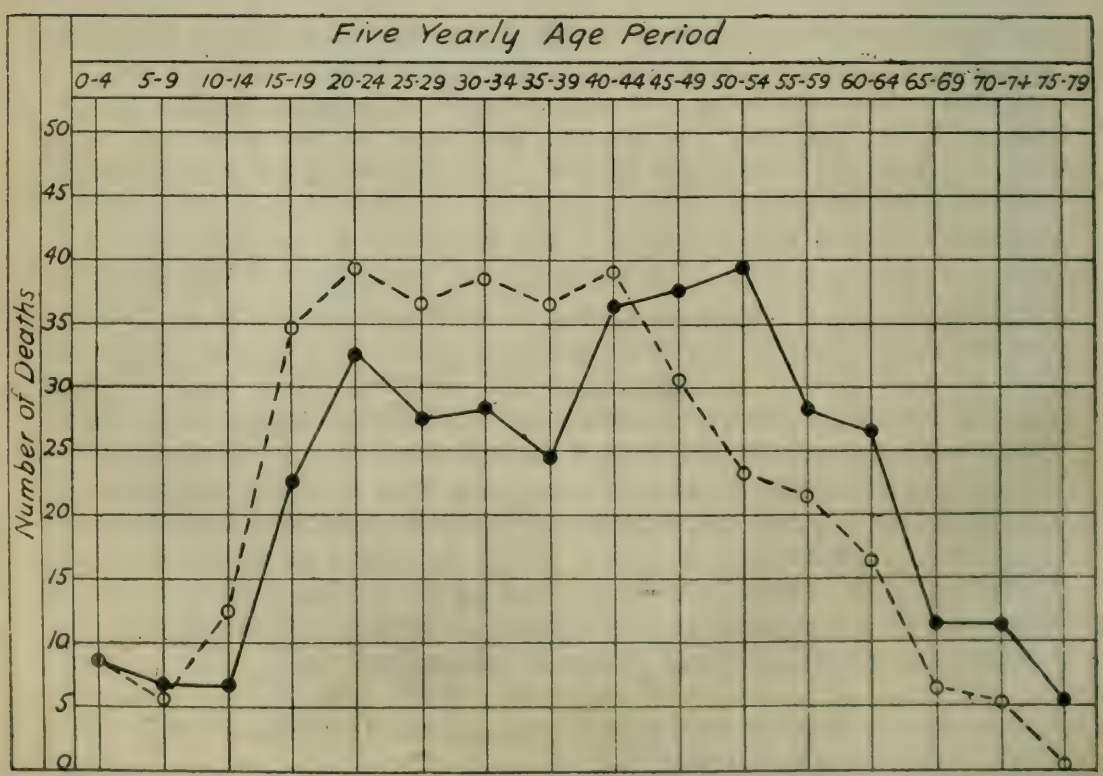


FIG. 27.—Male (continuous line) and female (dotted line) phthisis death-rate per 1,000 in Belfast during the war. A. Trimble.

TABLE XII.—*Occupations having Phthisis death-rate* 25 *per cent. above* (+) *or* 25 *per cent. below* (—) *the expected death-rate*, 1900-2, M. Greenwood.

Ref. No.	Occupations	35-45	55-65	35-45 (Dutch)
15.	Carman, carrier	...	...	...
16.	Bargemen, lightermen	...	...	—
18.	Dock labourer	...	...	—
20.	Farmer, grazier	—	—	—
21.	Farm labourer, farm servant	—	—	...
22.	Gardener, nurseryman	—	...	...
23.	Fisherman	—	...	...
24.	Maltster	—	...	...
25.	Brewer	...	...	...
28.	Stationery manufacturer	...	...	+
39.	Bookbinder	+	+	+
40.	Printer	+	+	+
41.	Watchmaker, jeweller	...	+	...
42.	Saddler and harness maker	...	...	...
43.	Butcher	...	—	...
44.	Miller	...	...	—
45.	Baker and confectioner	...	...	—
46.	Hatter	+	...	...
47.	Tailor	+	...	+
48.	Shoemaker	+	...	...
49.	Hairdresser	+	...	...
51.	Tanner	—	...	.
52.	Currier	+	...	...
55.	Gunsmith	...	+	...
56.	Lock-maker, gasfitter	...	+	...
57.	Blacksmith, striker	...	...	...
58.	Nail, anchor, chain-maker	...	...	...
60.	Bricklayer, mason	...	+	...
61.	Carpenter and joiner	...	...	+
62.	Slater and tiler	...	—	...
63.	Paperhanger, plasterer	+	+	+
65.	Cabinet-maker	+	...	+
66.	Sawyer	—	...	—
67.	Wood-turner and cooper	...	...	+
68.	Coach, carriage-maker	...	—	...
70.	Shipbuilding	...	—	—
71.	Chemical manufacturer	—	—	...
72.	Wool, worsted manufacturer	...	...	...
73.	Silk, satin, crape manufacturer	—	+	...
76.	Rope, twine, cord-maker	...	+	...
77.	Textile dyer, bleacher	...	—	...
78.	Carpet, rug manufacturer	...	...	...
79.	Hosiery manufacturer	...	+	...
82.	Glass manufacturer	+	...	+
90.	Coalheaver	...	—	—
91.	Gas-works service	—	...	...
92.	Platelayer, navvy	—	...	...
93.	Brick and tile maker	—	—	—
103.	Indiarubber worker	...	...	...
104.	Brush-maker, hair-bristle worker	+	...	...

The differences of mortality from phthisis separating different groups of the population are seldom realized. The following table is illustrative.

The comparative mortality figures for phthisis are from the Supplement to the 68th Annual Report of the Registrar-General:

Outdoor occupations		Indoor occupations	
Costermonger, hawker	516[1]	Inn, hotel servant (London)	669[1]
Messenger, porter (not railway)	368[1]	Inn, hotel servant	533[1]
Dock labourer	291[1]	Printer	790
Fisherman	96	Tool, scissors, &c., maker	353
Gardener, nurseryman	83	Brush maker	314
Farm labourer	82	Furrier, skinner	314
Agricultural	79	Chemical manufacturer	96[2]
Labourer in agricultural districts	82		
Gamekeeper	73		
Railway engine driver	62		

The next table shows the high mortality from all causes of merchant seamen compared with miners and farm workers. The seamen occupy close, over-heated quarters on board ship, while the coal miners work in well-ventilated mines and inhale coal dust, which exerts a stimulating action on the phagocytic action of leucocytes and excretory powers of the respiratory membrane. Merchant seamen and coal miners are both well fed.[3]

TABLE XIII.—*Death-rates from all causes per* 1,000 *living, Age Periods.*

	15–25	25–35	35–45	45–55
All occupied and retired males	3·5	6·3	10·9	18·7
Occupied and retired coal miners	3·8	5·1	8·0	15·2
,, ,, lawyers	—	4·9	7·6	13·8
,, ,, shopkeepers	3·1	5·6	9·4	16·4
,, ,, doctors	—	5·6	10·6	18·5
,, ,, farm workers	2·4	4·3	6·4	11·2
,, ,, merchant seamen	9·6	13·9	19·8	29·6

The 1911 census revealed in London 138,276 tenements each of one room. The population housed in them was 265,553; and of this quarter of a million persons 100,948 were children under 10 years of age. 32,351 of these one-roomed tenements contained more than two persons.

Mere statistics do not convey a vivid impression; I would therefore call attention to a singularly able paper by Dr. Wanklyn.

Dr. Wanklyn estimated in 1913 that 2,300,000 people in London are housed in tenements of one to four rooms. In the whole country it is estimated that eight million people are housed in one- to three-room tenements.[4]

A vast improvement has been made in the external environment of the dwellings so far as concerns water supply, drainage, scavenging. It is inside, not outside, that conditions urgently require reform.

Investigating a street of 'tenement' houses, Dr. Wanklyn says, 'the stairs and passages were filthily dirty; the sides of the passages were greasy, brown, and black, and although the front and back doors were open, and air was passing through freely, the house stank'. The accommodation of the top tenement in these houses is a front room

[1] Alcohol tells against these classes.

[2] The necessity of good ventilation and the slight irritating and expectorant action of fumes probably influence this class.

[3] The case incidence for tuberculosis was 2·59 per 1,000 for the British Navy 1908–13, 7·2 for the U.S.A. Navy 1914; 6·78 for volunteers for the Japanese Navy 1900–10. M. Paterson, *Shibboleths of Tuberculosis*, London, 1920.

[4] *Sect. of Epidemiology, Roy. Soc. Med.*, Oct. 24, 1913.

14 ft. by 11 ft. by 6 ft. 6 in., with a kitchen fire-place and a cupboard set in the wall, and a back room 9 ft. by 7 ft. by 6 ft. 6 in. which has no fire-place.

The woodwork is infested with bugs. There is no place for storing food or crockery except one wall cupboard in the front room. There is no scullery or sink, or even water for washing up, or any place in which to handle clean things; no water-closet nearer than in the back yard at the foot of 36 stairs, and that in common use by all in the house, no one responsible for its cleanliness. There is nowhere for the children to play. No coal or wood store, no cold-water tap nearer than the back yard. A man and wife and four children live in this place. Decency, privacy, personal cleanliness, quiet are impossible.

'For those who are ill-paid, ill-fed, and fagged out, the obstacles to decent living are too great, and the struggle is too unequal. They lose heart, they lose hope, they lose self-respect.' Hence the uncleanly conditions found in such tenement houses and the high incidence of disease and mortality.

The minimum requisites of a well-built cottage may be contrasted with the 'tenement' dwelling:

A living room and a parlour.

Three or four bedrooms.

A larder.

A scullery-washhouse which may contain a bath, with cold-water supply.

A water-closet.

A coal and wood store.

A cooking-range, affording a hot-water supply.

A drying ground for washed clothes.

A place for children to play.

In the tenement the coals are kept on the cupboard floor. The kitchen is represented by a gas-stove outside the door at the top of the stairs; the scullery by the cupboard wherein pots and pans were kept, a washing-up bowl on the table, and a slop-sink in the yard 35 steps below; the larder by the top shelf of the cupboard. The clothes are washed in a basement washhouse and hung to dry on a line stretched across the front room.

In the report of the Medical Officer of Birmingham it is stated that there are 47,000 back to back houses, and 200,000 people living in them, and that in these houses respiratory diseases, tuberculosis, and enteritis claim a far higher mortality.

Sir George Newman [1] found in Finsbury in 1906 that in a population of 101,000 the death-rate was 20·7 per 1,000. Sub-areas showed a death-rate following the social conditions from 8 to 36 per 1,000. Infant mortality varied 41 to 375 per 1,000 in these areas, the death-rate being the highest without exception where there was the greatest density of houses, the highest percentage of poor-class tenements, and a low standard of social life and domestic sanitation. The death-rate in four-roomed houses was 6·5 per 1,000, in three rooms 14·8, in two rooms 22·5, in one room 39. Similar figures, Sir George Newman says, hold good for other big towns.

It is to be remarked that the occupations subject to a specially

[1] *Med. Education in England*, 1918, col. 9124.

heavy incidence of tuberculosis are those the personnel of which are exposed to the domestic conditions of urban life. The improvement in urban amenities during the past decade—I am speaking of pre-war conditions—hardly extended to domestic life, they were extra-domestic. The late Frances Wood in her careful study of London statistics[1] inferred that between 1900 and 1911 'real' wages fell five or six per cent., i.e. that the rise in wages had not kept pace with the rise in the cost of living. Such are amongst the environmental conditions which operate upon the urban labourer in his home. Something remains to be said of factory conditions. In Table XIV I quote, after Collis, some comparative mortality figures.

TABLE XIV.—*Comparative mortality of certain classes aged* 15 *and upwards*, 1900–2.

Cause of death	Occupied and retired males	Shoe-maker	Publican, Inn Servant	Tin miner
Influenza	100	67	*171*	104
Alcoholism and liver diseases	100	77	*670*	28
Cancer	100	103	110	101
Phthisis	100	*145*	*173*	*436*
Other lung diseases	100	84	*148*	*419*
Nervous diseases	100	101	*178*	84
Circulatory diseases	100	100	*144*	105
Bright's disease	100	86	*243*	*143*
Accident	100	38	88	92
Suicide	100	100	*216*	32

The industries of printing and of tailoring show very similar comparative mortality data to those here stated for shoemaking. The same conditions pertain to these trades, viz., handwork in confined atmospheres producing nervous fatigue, and not entailing deep breathing or exercise of muscles which massage the belly and promote the circulation of the blood—conditions, too, which promote infection, for while cases of phthisis are able to continue at light work, they are eliminated from heavy-work factories. The third group—not an industrial group—represents the peculiar influence of alcohol plus an indoor life which entails little exercise. Neither exposure to cold nor exercise promote the rapid combustion of the alcohol consumed, and this acts then as a poison, not as a food. To a Scotch ghillie on the hills consumption of the same amount of drink may be relatively harmless.

'Industry', says Collis, 'supplies for the investigation of the causation of disease, great opportunities which are to-day hardly sufficiently appreciated, or, if appreciated, are not taken advantage of. Here are groups of people living under fairly similar housing and climatic conditions, but spending many hours of each day exposed to an environment entirely different according to the occupation they follow. Compare, for example, the working environment of a coal miner, a weaver, a cement maker, a shoemaker, a blast-furnace worker, or an agricultural labourer. The diversity is extraordinary, and far in excess of any alterations in environment presented by such things as housing, sub-soil, and climatic conditions which affect different towns

[1] *Journ. Roy. Stat. Soc.*, 1913.

and localities. Yet while the incidence of disease and especially of tuberculosis has been studied in relation to these latter conditions, it has not been closely investigated and correlated to the more diverse conditions of industry.'

The fourth group shows the influence of silica dust, which exerts a specific influence upon the influence of tuberculosis by damaging the lung tissue.

Tubercle bacilli are spread by droplets of saliva which are explosively scattered around as an invisible spray when we speak, sing, cough, sneeze. It is the inhalation of these droplets which is the danger, and of dust scattered from handkerchiefs first contaminated with sputum and then dried. Segregation of consumptives and anti-spitting regulations cannot eliminate this danger, for the saliva of incipient undiagnosed cases of phthisis may teem with the bacilli.

Infection with the bovine form of tubercle bacilli, e.g. through milk, causes surgical tuberculosis, but is of minor importance in the etiology of phthisis.

Romer asserts that most, if not all, tuberculous infection dates back to early life. The inquiry carried out by Hamburger and Monti in Vienna showed that 94 per cent. of all children aged 11 to 14 have been infected ; 87·7 per cent. of the children of Lille were found infected (Calmette).[1] The figures are less for a non-urban population, for example.

J. Parrot [2] determined the extent of tubercular infection by the cutaneous tuberculin reaction in *Arab children* to be :

	In villages		In tents	
0– 1 year	0		0	
1– 5 years	16·7	39·6 per cent.	20·8	30·5 per cent.
6–15 ,,	54·8		37·1	

in *Berber children* :

0– 1 year	0		0	
1– 5 years	11·9	25·6 per cent.	15·3	24·3 per cent.
6–15 ,,	48·2		31·3	

Doubt has been thrown on the validity of von Pirquet's tuberculin reaction, but confirmatory evidence of widespread infection has been obtained from the consecutive post-mortem examination of a large number of bodies. In most the infection is a mere temporary indisposition and protects against reinfection.

While massive infection may kill the 'unsalted' child or native, massive reinfection may lead to multiple foci.

The tubercle bacilli when inhaled are probably carried by phagocytes to lymph nodules. The primary tubercles develop in or near the dust-laden zone of the nodules. Thence infection spreads to lung tissue. The parasite must get out by making its way to the surface and so spread itself.

The ordinary simple normal life may be relied on to suppress tuberculous infection even before we are aware of its presence. Immunity established through infection is due to tissue reserves being called into action. The great underlying store of natural resistance must be kept up by normal hygienic living.

[1] 55 per cent. of children aged 5, and 95 per cent. of adults in New York.

[2] *Bull. Soc. path. exot.*, 1917, 10, 869.

Trudeau inoculated rabbits with equal doses of tubercle bacilli. Half of these were kept in open-air conditions, and half confined in a damp place to which sunlight had no access. On killing the rabbits at the same time after inoculation, the first lot were found to have slight, and the second lot extensive, tubercular lesions.[1]

Inflammatory and congestive processes may spread a localized infection, e.g. measles, pneumonia, whooping-cough, influenza. Severe physical exertion may disseminate the bacilli, e.g. parturition, athletic efforts. 'Confinement', says Theobald Smith,[2] 'in dark, damp, ill-ventilated rooms, depression of vital activity due to want of suitable food, and too low temperature are causes.' He pays no attention to heat stagnation as a cause. 'Recrudescence may be due to untoward conditions arising in modern life, which not only reduce general resistance, but also create in one way or another special places of lowered resistance.'[3]

He points out that 'all procedures for the cultivation of bacilli tend to modify these by tending to select and eliminate spontaneous varieties. The living tissues must similarly modify bacteria. Probably a change in the outer envelope occurs which either renders nutrition and multiplication more easy or the reverse.'

In Labrador and Newfoundland the mortality of the fisher-folk from tuberculosis is very heavy. It is generally acknowledged to be 4 per 1,000 of the population per annum against 1·52 for England and Wales. Some of the Labrador doctors talk of 7 and even 8 per 1,000 in certain districts. The general death-rate is a low one. The fishermen fish off shore, work for many hours a day in the fishing season, and live with their families on shore in one-roomed shanties. These shanties are built of wood, the crannies are 'stogged' with moss, and the windows nailed up, so that ventilation is very imperfect. They are heated by stoves and kept at a very high temperature, for example 80° F. Outside in the winter the temperature may be 30° below freezing. The women stay inside their shanties almost all the time, and the tuberculosis rate is somewhat higher in their case. The main food is white bread, tea stewed in the pot till black, fish occasionally, a little margarine, and molasses. The fish is boiled and the water thrown away. Game has become scarce in recent years; the old dark-coloured flour—spoken of with disfavour—has been replaced by white flour—that is, the flour has been robbed of its vitamines. In consequence of this diet beri-beri has become rife to a most serious extent. As to the prevalence of tuberculosis in Labrador we have to consider the intermarriage, the bad diet, the over-rigorous work of the fishermen, the overheating of, and infection in, the shanties. My informant, Dr. Wakefield, has slept with four other travellers in a shanty with father, mother, and ten children. In some there is scarce room on the floor to lie down. The shanties are heated with a stove on which pots boil all the time; water runs down the windows. The patients are ignorant, and spit everywhere, on bed, floor, and walls. In the schools the heat and smell are most marked to one coming in from the outside air. In one school 50 cubic feet per child is the allowance

[1] Cit. by Newsholme, *The Prevention of Tuberculosis*, London, 192.

[2] *Stud. Rockefell. Institute*, 1917, 27, 517.

[3] Tuberculosis did not increase in the armies of Britain, Belgium and U.S.A. during the war. Miller, *Am. Rev. Tub.*, Aug. 1919.

of space. The children eat irregularly throughout the day, and are kept in hot, close confinement. They suffer very badly from decay of the teeth. Whole families are swept off with tuberculosis, and the child who leaves home early may escape while the rest of a family dies. Here, then, we have people living in the wildest and least populated of lands suffering from all those ill results which are found in the worst city slums—tuberculosis, beri-beri, and decayed teeth.

The bad diet probably compels the people to conserve their body heat and live in the over-warm, confined atmosphere, just as pigeons fed on nothing but white bread, and so deprived of their vitamines, huddle to keep warm with their feathers out.

The warm, moist atmosphere lessens the evaporation from the respiratory tract, and therefore the transudation of tissue lymph and activity of the circulation through it. The unexpanded parts of the lung are not swept with blood. Everything favours a lodgment of the bacilli, and lessens the defences on which immunity depends. In the mouth the immunizing properties of the saliva are neutralized by the continual presence of food, and the temperature of the mouth is kept at a higher level, which favours bacterial growth.

Lieutenant Sein (Norwegian Navy) informed me that in northern Norway there had been an increase in tuberculosis. The old cottage fire-places with wide chimneys have been replaced by American stoves. In former days most of the heated air went up the chimney, and the people were warmed by radiant heat. Now the room is heated to a uniform moist heat. The Norwegians nail up the windows and never open them during the winter. At Lofoten, the fishing centre, oil motor-boats have replaced the old open boat. The cabin in the motor-boat is very confined, covered in with a water-tight deck, heated by the engine, crowded with six to eight workers. In these cabins the men sleep, eat, smoke, and spit. Knives, forks, spoons, dishes, and often pipes, they use in common. In the old days the fishermen used to occupy huts and walk about when in harbour; now they sleep and live in the motor-boat cabins. In old days they fished only in the spring and summer, now almost all the year round. In 1907–8 the mortality rate from phthisis at Lofoten was equal for males and females. In 1911 the mortality for males was more than three times that of the females.

The Norwegian fishermen feed on brown bread, boiled fish, salt mutton, and margarine, and drink, when in money, beer and schnapps: there is no gross deficiency in diet, as in Labrador, and beri-beri does not attack them. The one new condition which is common to the two districts is confinement and crowding in stove-heated, windless atmospheres.

Fishermen in sailing boats shut themselves up and sleep in cabins where the air not unoften gets so deoxygenated that the lamp goes out; yet they remain hard, healthy men. They balance the excessive exposure and hard work by day by heat-saving by night.

A correspondent writes, 'In my practice in the mining country of Lake Superior I have known eight Italians to sleep in a room about $12 \times 14 \times 8$ with the doors and windows tightly shut and still be strong, rugged men.' It is confinement unbalanced by open-air exercise that tells upon health.

It must be borne in mind that the cooling and drying power of the atmosphere are just as reduced in a stuffy seaside or country cottage, wind-sheltered by trees, other buildings, or the lie of the ground, as in the slummiest court of a city, and tuberculosis results therein no less than in the slum from lowered metabolism brought about by ill feeding, whether from poverty and ignorance, or a confined life coupled with exposure to infection in close cottage rooms. The prevalence of phthisis has been noted in convents, harems, barracks, and penitentiaries, places of close confinement.

Of 1,000 Russian Jews (80 per cent. of sedentary occupation) in the East End of London examined as recruits, 19·2 per cent. showed pulmonary tuberculosis, 13·5 per cent. other lung disease, 14·9 per cent. heart disease; 30 per cent. were fit and 30 per cent. of no military value. Of recruits examined in 1917 in London, 3·1 per cent. had tuberculosis, 9·9 per cent. heart disease; 1 in 8 were unfit from these two causes.

The Kalmucks geographically and linguistically are a nomadic race destitute of even the primitive principles of hygiene, and dirty and verminous. Among them syphilis, small-pox, alcoholism, tuberculosis are said to be rampant. The effect of contact infection is shown by the fact that tuberculosis mortality is very great, particularly among the young adolescents, where the Kalmucks come in contact with the Russians on the frontier. Not with the Kalmucks, the Lofoten fishermen, or the Labrador folk can a spacious and pure open-air environment balance bad feeding and close contact with carriers in sleeping quarters.

'Phthisis', says Sir Harry Johnston, 'kills as many negroes or mulattoes as it does Caucasians; pneumonia is still more deadly in the negro community.' The mortality from tuberculosis among coloured males in the age-group 25 to 54 was 4·77 per 1,000 according to the experience of the Metropolitan Life Insurance Company, New York.[1]

In Rio de Janeiro the tuberculosis mortality is high, falling from 12·2 to 5·4 per 1,000 from 1863–91. In the whole Federal district the mortality was 4·61 per 1,000 in 1915.[2]

Dr. Angus Macdonald[3] reports that, 'except in Australia where the rate was only 1 per 1,000, and in Grenada 0·5 per 1,000, the death-rate from all forms of tuberculosis is considerably higher in most tropical countries than in the British Isles, ranging from 3·4 per 1,000 in Jamaica and Ceylon to about 5 per 1,000 in Trinidad and British Guiana. Pulmonary tuberculosis caused 84 per cent. of deaths, other forms only 16 per cent., pointing to the comparative rarity of the bovine tuberculosis'.

'Occupation', he says, 'played little part in the extension of the disease. Food infection had little or no effect, as tuberculous meat was rare, and milk was practically always boiled. Let it be noted that it was consumed in relatively small amount. Climate, as a causative factor, was not statistically evidenced, the mortality rate in the damp and relaxing climate of Grenada being 0·5 per 1,000, while

[1] L. L. Dublin, *Mortality Statistics of Insured*, New York, 1919.
[2] Barrosa, *Brazil Medico*, 1917, July 28, Aug. 4 and 11.
[3] *Brit. M. J.*, Aug. 23, 1913.

it stood at 3·4 in dry and bracing Kingston. Evidence from everywhere pointed to tuberculosis being a disease almost entirely of domesticity. The wretched hovels of the people, the overcrowding of rooms at night, the closing of doors and windows and stopping of every chink, absence of ventilation and fear of night air, the fatalism of the people—all these features in tropical life enhanced the opportunity of infection and made inevitable direct communication of the disease from an affected person to all within striking range.' 'The chief causes of death in the tropics, far from being purely tropical disease, were the time-honoured diseases of civilization.'

According to B. Moore the two main factors, apart from special causes such as silicosis, causing phthisis are the long-continued strain of factory workers under unhealthy conditions, and the infection of such, so reduced in resisting power, by workers suffering from phthisis. The heaviest incidence is, he says, among those occupations which, demanding slight muscular exertion, allow the phthisical to continue at work in crowded factories and disseminate the bacillus, e. g. among printers, boot- and shoe-makers.[1] In the West Midland region, out of 319 cases of phthisis among men examined for the army 132 were unaware of their disease. In the Leeds area, among 831 men rejected as recruits for phthisis there were 6 bakers, 66 clerks, 7 hairdressers, 83 metal workers, 2 milk dealers, 30 mill hands, 10 schoolmasters, 60 shopkeepers, 145 tailors.[2]

The lighter, sedentary occupations are carried out in closer atmospheres, and do not entail deep breathing, increased metabolism, and other advantages of more strenuous labour. Lawyers have a low mortality rate for tuberculosis, but they balance their long hours of sedentary occupation by holidays and exercise, they feed well, and seek immediate treatment when ill.

Attributing the greatest importance to infection, national colonies for segregating consumptives have been proposed by Esslement.

There should be workshops attached to a sanatorium, or village colony, for each trade, in which the phthisical might be brought back to work gradually as their cure progressed, and in which the incurable but chronic cases might continue to work segregated from the healthy.

Brownlee[3] regards phthisis pulmonalis not as a single disease, but as a group arising from differences in the various strains of tubercle bacilli.

The differences, however, between early and middle-age phthisis may not be one of strain of bacillus but of humoral effect of the body. Young adult phthisis is probably correlated with strain of work, ill-feeding, and exposure. It is common among seamen, dock labourers, and agricultural labourers. Middle-age phthisis is correlated with density of population and with tuberculosis in children. Old-age phthisis is associated with miners and workers in slate quarries.

He thinks 'a considerable part of the decline of phthisis in recent years is in line with the biological properties of diseases in

[1] The case of tuberculosis among the sick-berth staff in the Navy were 6·43 per 1,000 of average ratio borne, 1908-13, and 2·24 per 1,000 among the seamen. M. Paterson.

[2] *Rep. on Phys. Exam. of Men of Mil. Age.* [Cmd 504.]

[3] *Epidemiology of Phthisis, Medical Research Committee, Special Report Series*, No. 18, 1918, and No. 46, 1920.

general, e.g. with the variations in the life-history of infecting organisms, and has little to do with hygienic conditions'.

He further says, 'There seems no statistical evidence that the young adult type of phthisis is affected to any extent by environment. In this respect it would seem to resemble scarlet fever, as in the case of this disease there is very little proof that in any individual city it is more common in the more crowded parts than in the better-class parts of the town.'

'The number of cases of this type which occur in eminently healthy houses is quite surprising.'

I would point out that children of the well-to-do are kept indoors for fear of catching cold, and at sedentary occupations, cramming for examinations, &c., and are often wrongly fed or over-indulged. They may suffer from the conditions of environment dealt with in this report no less, and perhaps even more, than children of the poor who play in the streets, and are less forced in education. Middle-age phthisis is correlated with hygienic conditions. Old-age phthisis particularly affects miners and workers in slate quarries.

The economic importance of eliminating tuberculosis is illustrated by the fact that the Rand mining industry paid in the five years ending July 31, 1917, £2,850,012 as compensation under the Miners' Phthisis Acts, and in contributions towards the Miners' Phthisis Sanatorium.

The birth-rate has declined to the extent of one-half in the last 40 years. In the three years ending 1917 it fell from 23·8 to 17·8, just over 25 per cent.[1]

T. H. C. Stevenson[2] has shown that fertility decreases as the size of the tenement increases till six or seven rooms are reached; that the infant mortality decreases regularly as the size of the tenement increases, being for tenements of ten rooms and more less than one-third of that in one-room tenements.

The more prosperous classes are largely limiting their children by artificial means of preventing conception. Apart from this, conditions of city life tell against fertility.

Those who spend much time actively engaged out of doors, provided the food is adequate, are most virile and fertile. Those who suffer most from nervous instability are the sedentary people who spend their time in stagnant atmospheres indoors. In the tropics it is the white women who particularly suffer in health and lose fertility.

Sterility is common among milch cows wherever large numbers are brought together and intensively fed for breeding and heavy milk production. The higher the dairy development of the cow and the greater the restraints of an unnatural environment the more the infertility.

Lack of balance in the ration, under-feeding or gross over-feeding, increases sterility. Of 24 young men fed on a low war ration, 22 acknowledged they had lost sexual desire. The lack of the food-accessory bodies leads to atrophy of the sexual organs and sterility in pigeons.[3] The divorcement of the city population from gardens and fresh green

[1] G. Newman, *The Outline of the Practice of Preventive Medicine*, 1919. [Cmd. 363.]
[2] *The Declining Birth-rate; its Causes and Effects*, 1916.
[3] R. McGarrison, *Indian J. M. Res.*, 1919, 6, 275.

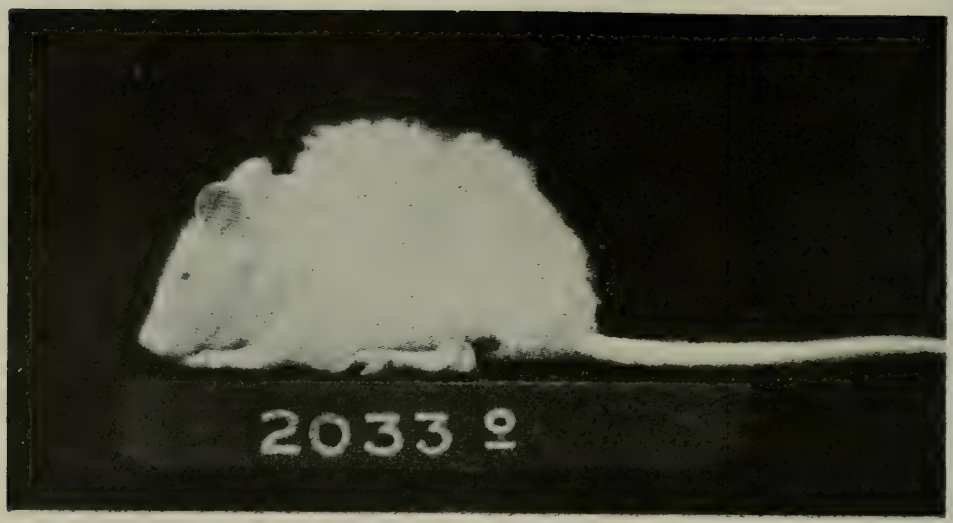


Fig. 28. Rat at age of 505 days, after prolonged stunting at body weight of 53 grm. by limiting the daily quantity of food, which was sufficient in quality. Osborne and Mendel.

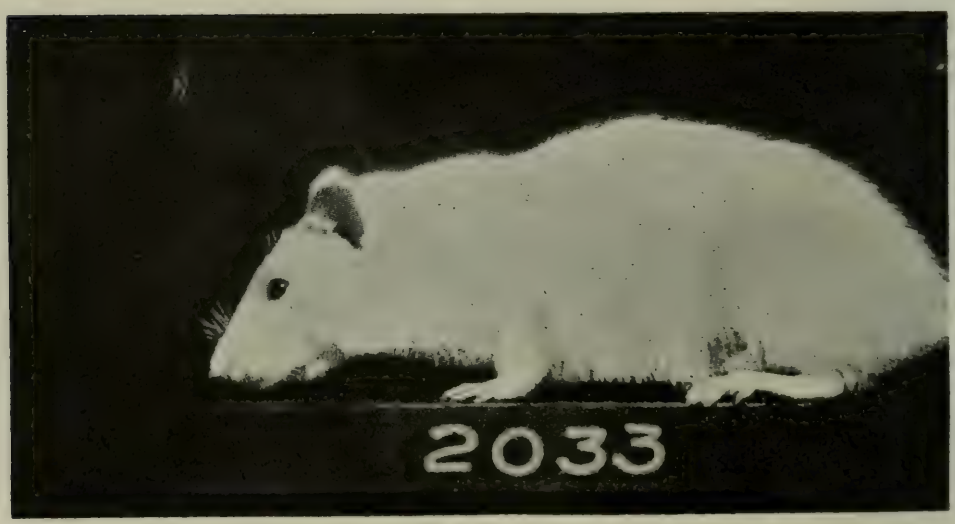


Fig. 29. Resumption of growth of rat to 205 grm. on full diet. Such a rat breeds later, and may live longer than normal. Osborne and Mendel.

foods, lack of sufficient dairy produce in the diet, and the millers artifices of removing the germ from wheat, &c., probably lead to infertility.

The Ulster spinning-mill girls have taken to a diet mainly consisting of white bread, cakes, sugar, and tea. The question arises whether workers living a celibate life instinctively take to a diet which depresses the sexual functions.

Osborne and Mendel [1] find, while yeast will supply the necessary vitamines to the artificial diet used by them for feeding experiments, it fails wholly to fulfil the development of the sexual glands, their animals, otherwise well-nourished, remaining sterile. This opens up a new field for exploration.

The influence of rural conditions in keeping down the infant death-rate is seen in the following figures for 1914:

	Deaths under one year per 1,000
England	
Twenty-eight leading cities or urban districts	140
County boroughs of the north (industrial)	130
Rural districts of the south	66
Ireland	
Towns with population over 10,000	120·7
Eight western counties	50
France	
Towns with population over 5,000	111·4
Rest of France	57·8

Sixty to seventy was the actuarial standard death-rate for infants fixed by Ansell for the peerage about 50 years ago. In Dundee the infant death-rate is under 50 among the professional class, and 150 for the whole town. Taking 50 as a standard, 60,000 infant lives were lost in 1914 from preventible causes.

How much can be done to reduce child mortality in a decade or two is shown by the fact that the deaths per annum under five years for England and Wales were 106,000 in 1900–2, 79,000 in 1910–12, and 63,500 in 1917.

Fifty years ago mortality from fevers formed almost 5 per cent. of the total mortality of the country. It now forms an almost negligible quantity.

In Petrograd (1906–15) the rate was 122 in the better quarters and 279 in the industrial quarters. C. E. A. Winslow,[2] who investigated the infant death-rate in Russia in 1917, says there were a million and a half infant deaths every year, and two-thirds of these were preventible. Seventy per cent. of the men and 96 per cent. of the women are illiterate among the true Russians.

In Bavaria the infant death-rate is higher in the rural districts than in the towns; the town population are better instructed in hygiene. The mothers in rural districts go out to work all day. In Danzig (1915–17) breast-feeding was encouraged, and welfare work instituted; the rate fell from 177 to 109 among legitimate infants in spite of the continuance of the war.

Welfare work appears to have lowered the rate in New York from

[1] *Year Book Carnegie Institute*, 1918, 302.
[2] *Public Health Rep.*, 445, Washington, 1918.

144 in 1907 to 88·8 in 1917, in Marylebone from 190 in 1899 to 90 in 1914.

The rebuilding of a slum area in Liverpool lowered the rate from 300 to 167.[1]

In 1901 the death-rate of infants attending a school for mothers at Ghent was 260. With the use of dried milk for artificial feeding, and domiciliary visits of welfare workers, the rate fell to 34 in 1908.

The tenement baby is born healthy and of good weight; it is the food and environment that decides its mortality. High infant mortality is due to excess of respiratory and digestive ailments, particularly of the latter. The tenement baby is over-clothed and confined indoors by its mother for fear of its catching cold, and to save trouble under the difficulties of tenement life. It suffers from lack of adequate cooling and evaporative powers of the atmosphere, and of exercise. The baby when fed artificially is not only fed, in many cases, on wrong food, or food deficient of vitamines, but is often over-fed. While its immunity

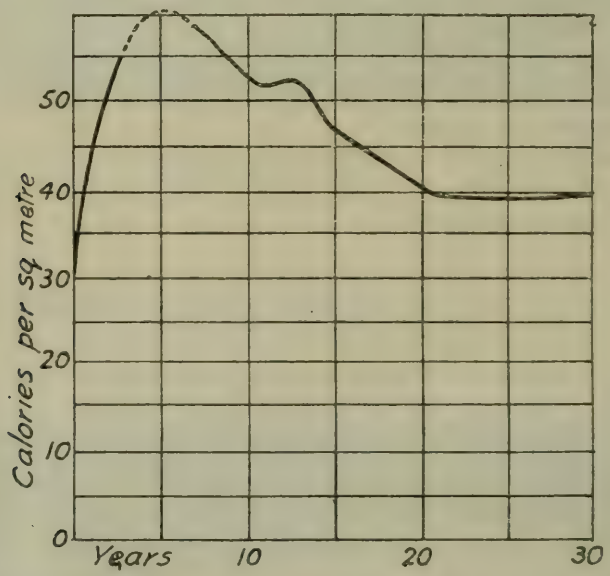


Fig. 30.—Curve showing that the metabolism of infants per sq. metre of surface increases during the first year (Graham Lusk). In the case of a baby of a wild man the metabolism is probably highest very soon after birth. The civilized baby is over-coddled.

to infection is lowered by bad feeding, the close, humid air of the tenements particularly lowers the defences of the respiratory membrane by lessening the flow of blood, lymph, and secretion through it.[2]

The Royal Commission of Scotland on Physical Deterioration of the Race reported that 50 per cent. of the town children were affected with adenoids or other pathological conditions of the nose and throat. The explanation is, I think, to be found in the close, dusty air of rooms, which, while increasing infection, diminishes metabolism and vigour, and lessens the natural cleansing mechanism of the respiratory membrane.

The death-rate of infants from digestive disturbances is greatly

[1] In Dresden (1886) 18·49 per cent. of infants died under one year in low-lying, shut-in courts, 2·5 per cent in the high-lying and open Kieferstrasse, none in the model houses of the Johann Meyer-Strasse (Meinert).

[2] See Part I of this Report, *Medical Research Committee, Special Report Series*, No. 32, p. 150

increased by hot summers, the heat lowering the infant's metabolism and increasing its thirst; thus it is over-fed if on the bottle given food when it wants water; the heat also increases bacterial decomposition of the milk.

The temperature coefficient of bacterial multiplication is 2 to 3 for 10° C., and as the multiplication proceeds logarithmically a small change in rate suffices to produce a large difference. Thus if a number *n* of *B. coli* be inoculated in milk at 60° F. and 70° F. respectively, the number after twelve hours will be 126 *n* and 4,000 *n* respectively.[1]

Dust and flies ensure the contamination of milk in hot summer weather. The heat favours the dissemination of pathogenic germs outside the body; the sun cannot enter the slum tenements and exert its beneficent sterilizing action on dust and dirt.

E. Mellanby finds that while a diet deficient in fat-soluble *A* vitamine produces rickets in puppies, mere confinement on an adequate diet will not do so. On the other hand, Findlay, Paton, and Watson found puppies on a diet on the border-land of deficiency became rickety when confined and not when kept in open-air conditions. Babies are not only confined, but kept smothered with clothes in a warm, moist environment. If the diet is barely adequate in growth principles, and the metabolism is lowered, and the digestion disturbed by environment, dietetic inadequacy must result.

E. Pritchard has weighed slum infants before and after breast meals, and finds the mothers afford but small feeds. These, he says, are adjusted to the needs produced by the close atmosphere in which the infants live.

The offspring of rats, fed on an inadequate amount of fat-soluble *A* vitamine, may show no obvious sign of malnutrition but an unusually low resistance to infectious diseases, which only improves when the young rats are given a richer supply of this necessary factor. In puppies the rachitic condition need be little advanced before the animals become lethargic, and far more liable to be infected by infectious diseases such as mange, broncho-pneumonia, and distemper. Ninety per cent. of the negro children in the Columbus Hill district of New York are said to be rachitic. Cod-liver oil was given, and of those babies who had 54 oz. in 6 months, 93 per cent. were non-rachitic; and of those who had 21 oz., 58 per cent.; of those who had none, 6 per cent. The adequate supply of the three food-accessory bodies—water-soluble *A* and *B* and fat-soluble *B*—is of most vital importance to children.

As the result of under-feeding at Lille during the war 60 per cent. of the children showed arrested growth, and 40 per cent. tuberculous glands.

In the choice of diets for tubercular patients foods are particularly included which are now known to be rich in vitamines. Of no less importance is a wide enough choice of protein, animal as well as vegetable, ensuring a supply of all essential amino-acids. Milk and butter fat are essential foods for children, and their place in the diet must not be replaced by margarine and seed products.

In Glasgow 50 per cent. of the poorer class of children are affected with rickets, the age incidence is six to eighteen months, the

[1] J. L. Claypon, *J. Hyg.*, 1909, 2, 239.

development of the disease takes place towards, or at the end of, the winter. Diarrhoea was present in 33 per cent. of the rachitic and 11 per cent. of the non-rachitic. To neutralize acid products of bacterial decomposition in the bowel basic elements such as calcium may be withdrawn from the body.

There was a far lower percentage of rickets among children taken out of doors for exercise than among those not taken out. In the garden cities, St. Helens and Bournville, there is very little rickets.

The school medical officer of the county of Salop,[1] says 'amongst the children of the elementary schools the most neglected have the least caries'. The latter, in country villages, get more raw vegetable food such as turnips, and less sugar and white bread. They also live a more outdoor life. Caries is associated with rickets and vitamine deficiency. Tooth-brush drill is not a preventive.

In the open-air schools of the L.C.C. established for weakly children, where good food, rest, and work in the open air are provided, the weight and health of the children increase amazingly. Cases of heart disease, nervous children easily overwrought, those in danger of tuberculosis, those subject to colds and coughs, are alike benefited beyond measure. There are now 120 such schools in New York.

I cite the case of a home for tubercular children taken from slum tenements, where, unless there is a bitter wind, the children are more or less uncovered. In the winter, after the baths, they run round the sleeping shelter with bare legs and scant attire. In summer weather they go about all day with only knickers on and sleep in canvas shelters. They never show discomfort from cold, or suffer from catarrhs, and come to regard going in as a punishment. Well fed to meet the energy demands, they do extraordinarily well. Going back to the slums they relapse again, becoming thin and pale from the caged life and ill-feeding.

Children of city schools lack playing grounds and open-air exercise, and training in character by games is not made the first, but the least important, part of their education; there pertains a system of punishment by confinement indoors and the writing of lines, in place of punishment by compulsory walks or runs which would make the child good-tempered and amenable to discipline. Clever students are rendered feeble in physique and neurotic in temperament by the system of cramming for examinations and long hours of home work.

I cite the case of a girl who suffered from a constriction of the larynx following cauterization which so hampered her breathing that she could take no exercise without loss of breath, and in consequence became thin and drawn looking. After tracheotomy she walked miles, gained a pound in weight a day, and quickly reached robust health. Schoolchildren are no less starved in health by home work and cramming for examinations, and the vigour and happiness of thousands is thus sapped.

'I would consider', says Almond of Loretto School, 'the Chinese punishment of depriving of sleep as less dangerous than the depriving of exercise. It would be much better to put a boy on bread and water than do that.'

[1] Sims Wallace, *Child Welfare*, &c., London, 1919.



Fig 31. Open-air treatment for tubercular children. Mrs. Brenton's Home.



Fig. 32. A baby of Lieut. Muller's hardened by a period of daily exercise, while naked, in the sun and open air.

There is no question of the greater health of the countryman, and yet he is not the best stock, for the better class seeks advancement in the towns; moreover, he receives the lowest wage[1] and sustains himself and his family in insanitary cottages on the cheapest food-stuffs. These cheap foods, however, include enough fresh vegetables, and, in spite of the towns robbing him of milk, he thus secures a sufficiency of the food-accessory factors. The open-air exposure, muscular exercise, adequate rest, and absence of nervous exhaustion ensure appetite and digestion in the countryman, and prevent intoxication by bacterial products of digestion. If he were better fed and housed the superiority of his health would be still more marked.

The city workers are occupied for long hours at monotonous work in shops, offices, and factories, in close atmospheres, often artificially lit. The rush and strain of competition sharpen the wits, but nervously exhaust them. Their hours of recreation are devoted to the still warmer and windless atmosphere of the meeting-house, public-house, music-hall, or cinema palace; they travel in the confined atmosphere of the tube railway carriages and lifts, or trams. They have no outdoor exercise or games. The men medically examined for the army in 1917 in the West Midland region were described as middle-aged at 30, old at 40.

As shown by the evidence given in the first volume of this report, the indoor atmospheric conditions of offices, shops, business houses, and factories closely approximate to the outdoor conditions which pertain to such a climate as Madras and Sierra Leone, a climate which is recognized to be enervating to white men. The stillness of the air in confined spaces has a most potent influence on the cooling functions of the skin, on the cutaneous nerves, and so on the vigour, health, and efficiency. The citizen by his indoor life, and by the smoke and dust of cities, is withdrawn not only from the influence of wind but from the influence of the sunlight, the radiant energy of which has undoubtedly a most potent influence on the cutaneous nerves and feelings determined by these, and probably a no less important influence on the blood and other tissues exposed to its action. He is exposed to artificial methods of heating and lighting.

In the winter, six months of the year, the life of a city clerk or shop employee may be something like this: After rising in the morning, breakfast and a brief walk to the station in the open air; from 8.30 to 9 in train—in winter a crowded, shut-up, and confined space—from 9 a.m. to 1 in an office or shop, with windows opening maybe on to a well and artificial light on all day, heated by so-called radiators, which produce a relatively humid, warm, stagnant atmosphere round the head, the floor being more or less cold; from 1–2 p.m. in a tea-shop with a brief walk to and from it, the tea-shop being often underground and lit with artificial light and having a humid, stagnant atmosphere; from 2 p.m. to 5 or 6 p.m. again in the office or shop; from 6–6.30 p.m. again in the train or tram: a brief walk home followed by leisure spent at home, in the meeting-house, cinema, or music-hall, by the few who are not too tired in a spell of open-air exercise, and so to bed. Within doors, then, in confined, artificially

[1] In Oxfordshire wages were as low as 12*s*. a week before the war.

heated and often artificially lit atmospheres, almost the whole 24 hours. Is the winter deterioration in health to be wondered at, or the epidemics of colds which sweep through the community?

When the citizen takes his walk in the streets, motor-cars pollute the air with exhaust fumes and in dry weather act as dust squirts, making the atmosphere teem with dust particles, made unclean by man and animal. Missing the exhilarating stimulus of the sun and cool, moving air, he repels the dull uniformity of existence by tobacco and by alcohol, or by over-indulgence in food, for example, sweets, which are everywhere to his hand, and by the nervous excitement of business and amusement. He works, he eats, and is amused in warm, windless atmospheres, and suffers from a feeble circulation, a shallow respiration, and a disordered digestion.

In the report on the medical examination of recruits in the Eastern region it is stated, 'to any one who has examined recruits from the various races of India, the prevalence of disease and defective physique among the young men of Great Britain presents a shocking contrast. Amongst the large number I examined from time to time in India I cannot recollect a single example of pulmonary tuberculosis, heart disease, hammer toes, or pes cavus.'

It is healthy and happy conditions of life, not higher wages, which require to be secured. Higher wages will not bring these conditions to people housed in tenements and separated from all means of rest and play in natural surroundings.

How powerful is the influence of exercise in the open air, coupled with rest, good feeding, and a disciplined life, is shown by the change of millions of relatively pale, feeble, nervous, and anxious clerks, shop or factory hands, into the ruddy, healthy, bigger, and more muscular and magnificently fighting soldiers of the British army.

The garden cities of the England reconstructed should be small cities modelled in the manner of Adelaide. In the centre are the places of organization, business, shopping, meeting, education, and indoor amusement, surrounded by a ring of dwelling-houses and their gardens with workshops intermingled. Tramways run radially from the centre and not only serve for business but convey the population to the outer wider country on holiday expeditions. The citizens' workshops are lit by sunlight and well ventilated on every side; electric power is supplied and smoke is absent. The citizens can walk to and from business in the open air and not be exposed in the least degree to the carriers of disease; have their meals at home or in airy canteens supplied with well-chosen and well-cooked food; spend part of their leisure each day in the playing fields or garden, with free access to the beauty of nature. Disciplined as children in civic duty and in the art of living, industrial workers may attain to a much higher level of happiness. It must be recognized that discipline ordered by knowledge of the true principles of health is the basis of improvement in general welfare.

CHAPTER VI

Fever and Open-Air Treatment.

According to Pfluger the oxygen use increased in curarized animals 10 per cent. for each degree as the body temperature was raised by a bath from 39° to 41° C. In normal animals the increase was 5·7 per cent. Rubner found that while a bath of 37° had no effect on the metabolism of man, one of 44° increased the volume of respiration 18·8 per cent., the oxygen absorption 17·3 per cent., and the carbon dioxide elimination 32·1 per cent. As the polypnoea excited by the heat washes carbon dioxide out of the body it would produce a certain degree of alkalosis, were appropriate adjustments not made in the body and by renal excretion.

There has been much discussion as to whether fever is due to increased heat production or diminished heat loss. Exact calorimetric observations have settled the question. In typhoid fever there is an increase both of heat production and of heat loss, but as the latter does not balance the former the temperature rises.[1]

The onset of fever was studied calorimetrically by Benedict and Carpenter in the case of a subject who was exposed to mercurial poisoning arising accidentally from mercury valves used in the ventilation current of the calorimeter. Thermogenesis increased 15 to 30 per cent., and thermolysis failed to balance this :

Time	Heat lost Calories per hour	Heat produced Calories per hour	Body temperature ° C.	
5-7 a.m.	73·4	79·2	36·53	
7-9 ,,	92·4	97·1	36·90	Subject making bed.
9-11 ,,	91·8	106·0	37·15	Malaise begins.
11-1 p.m.	87·7	112·0	38·00	
1-3 ,,	79·6	108·0	38·19	

Barr and Du Bois [2] studied the onset of malaria chill in a patient placed in a calorimeter. There occurred a great increase of heat production with no increase of heat loss ; after the chill stage the heat loss increased, and chiefly by sweating. In the cold stage the diastolic pressure rises greatly owing to vaso-constriction, to fall greatly in the hot stage and return to normal in the sweating stage.

The skin temperature of the abdomen during rigors may be 7–11° F. below the rectal temperature.[3]

While an increase of body temperature by baths seems to have no such effect, infectious fevers are characterized by a destruction of tissue protein and wasting of flesh.

The ingestion of a large amount of carbohydrate with a moderate protein diet may maintain a typhoid patient in nitrogen equilibrium,

[1] Coleman and Du Bois, *Arch. Int. Med.*, 1915, **18**, 887.
[2] *Arch. Int. Med.*, 1918, **21**, 627.
[3] Palmer, *Guy's Hosp. Rep.*, 1911, **45**, 103.

'the high caloric diet' of Colman and Schaffer. The specific dynamic action of food in typhoid fever is almost absent.

Parenchymatous and fatty degeneration are not produced in rabbits by keeping them at a higher temperature than normal. These degenerations are due to toxin, not to temperature. (G. Lusk.)

A considerable rise of temperature occurring in the first stage of typhoid fever is a far more serious symptom than in a relapse. The toxin which affects the heat-regulating mechanism need not be the same then as that which poisons the tissues. (Brownlee.)

Mandel[1] suggests that the purin bases increased in fever and perhaps liberated through the destruction of tissue may play a considerable part in producing fever, acting on the heat-regulating apparatus of the mid-brain. Fever can be produced in monkeys by injecting xanthin. The giving of salicylates prevents this.

That tissue protein contributes to the production of heat in fever is shown by the increased nitrogen output and waste of muscles. The ammonia, uric acid, and kreatin percentage output increases, while that of urea diminishes.

It is said that fever cannot be produced by a toxin if the mid-brain be severed from the spinal bulb. A man with the spinal cord divided in the upper dorsal region is cooled by exposure and warmed by hot bottles in bed as a cold-blooded animal would be. His thermal regulation is paralysed below the point of section.[2] The decerebrate animal has no heat-controlling mechanism, and must be kept in a bath at 38·5°–39° C.[3]

Some evidence has been brought forward to support the theory that fever is a protective mechanism. The streptococcus of erysipelas does not develop at 39°–40° C., and is killed at 39·5°–41° C. Anthrax bacilli kept at 42°–43° C. for some time became attenuated, pneumococci at 41°–42° C. The attenuated anthrax bacilli when injected confer immunity. Hens, with their high body temperature, are immune to anthrax; frogs become susceptible only when their body temperature is raised; there is, then, an optimum temperature for anthrax. The object of the increased heat production and the cutaneous vaso-constriction in the 'chill' stage of a fever may be to combat the infective process by augmenting the flow of blood through the infected organs and accelerating the rate of chemical reaction.

While exposure to high air temperatures (over 35° C.) by producing a condition of fever may hasten the production of antibodies, exposure to moderately high temperatures (30°–35° C.) apparently tends to decrease the power of producing antibodies, probably by diminishing metabolism.[4]

To moderate fever wetting and fanning the body are measures of the greatest value, relieving the patient by raising the vascular tone, lessening the frequency of the heart, and so increasing the oxygen supply, augmenting diuresis, &c.

Dr. Francis Hare writes that in 1887 at Brisbane Hospital 'Systematic cold bathing was introduced and applied to every typhoid

[1] *Am. J. Physiol.*, 1907, **20**, 439.
[2] Kennaway and Pembrey. *J. Physiol.*, 1912, **45**, 82.
[3] Bazett, *Proc. Roy. Soc.*, July 12, 1919.
[4] C. E. A. Winslow, *Proc. Soc. Exper. Biol. and Med.*, 1916, **13**, 194.

patient admitted (except when clearly contra-indicated); and this has been continued with modifications down to the present time. Very soon after the change it was found that much less alcohol was being used, because the circulatory conditions demanding it had become much less frequent.

'The guide for the number, duration, and coldness of the baths was the amount of temperature reduction which followed bathing. The main advantage of systematic cold bathing resides in its power to anticipate and prevent the progressive vasomotor paresis, which in some degree is an invariable accompaniment of all continued pyrexia if at all prolonged, what is commonly referred to as cardiac failure being in reality failure of vascular tone.'

The polypnoea in febrile patients is lessened by keeping down the temperature by open-air treatment; thus in the phthisical the lungs are given more rest. In the hot season in the tropics many fever patients die of heat-stroke owing to the incoordination of the heat-regulating mechanism—one of the chief reasons, according to Woodruff, why Europeans are killed off in the tropics.

Fevered patients ought to be treated in open shelters or verandahs. In warm weather they can be blown upon by fans or 'punkahs' to promote evaporation, and if their skins fail to sweat enough, their garment can be damped with a water spray or water-dripping arrangement. In the tropics the institution of such methods of treatment is imperative, and the exact observation of their effect on temperature, pulse, and respiration require to be recorded.

Evelyn, in his diary, records the death from 'a quartan ague' of his child, an infant prodigy who could write in four languages at the age of four. 'In my opinion he was suffocated by the women and maids who attended him, and covered him too hot with blankettes as he lay in a cradle near an excessive hot fire in a closed room.' What a procession of unfortunates have been thus killed by their attendants acting under erroneous fears of chill.

The provocation of sweating and exposure to a gentle draught is the old method correctly based both on the effective physical laws of cooling by evaporation and convection and the physiological reactions of the skin. By a hot bath or warm sponging the blood is brought to the skin and then cooled by evaporation and wind. The Japanese place the patient in a hot bath 104°–107½° for 5–7 minutes and then back to bed.

Immersion in a cold bath, on the other hand, constricts the blood-vessels, and, while cooling the epidermis, may actually raise the rectal temperature. The epidermis is a very bad conductor of heat, no less so the subcutaneous fat. Taken out of the bath there will be little loss of heat from the chilled skin which does not sweat and has its blood-vessels constricted.

It is probably advantageous to immunity that evaporation freely takes place from the skin and respiratory membrane. As a result immunizing substances may pass, not only through the skin and membrane, but into the blood with the tissue fluid which makes good the blood volume. The old treatment of bleeding carried out by observant and able men cannot have been founded on ignorant custom. Bleeding draws tissue juices into the blood in large quantities, and

these juices may well carry from the tissues potent immunizing properties. The determination of blood to the skin and free sweating may no less contribute to the immunizing powers of the blood, for the loss of water by sweating must be made good from the tissues when drink is not available; thus the blood undergoes a large degree of change, giving and taking up fluid. Exercise carried to the point of sweating, but without exhaustion, is for this reason among others advantageous to the non-febrile. Sweating as a treatment of fever does not merely act by cooling the body by evaporation, but by the increased interchange set up between the blood and tissue fluids. Diuresis and purgation will act in the same way. Acetylsalicylic acid (aspirin) in 1 grm. doses, while having no such action on normal persons, increases the heat-loss of the febrile nearly 40 per cent., and thus lowers the temperature 1·5° F. The drug causes loss by sweating, scarcely influencing heat production.[1] A dose of 30 grm. of dextrose lowers a febrile temperature 0·5°–2° C. by increasing heat-loss.[2]

The open-air treatment of phthisical patients requires to be based on physiological principles, not on a blind belief in the virtues of open air. The patient needs the conditions which will most promote his health and happiness; he does not want to be chilled and made miserable. The first essential in treatment is a cheerful environment, giving present content and hope of future cure. The wild animal seeks shelter from the wind, and a warm lair to couch itself in. It is as unreasonable to expose a debilitated fevered patient to wet, cold, windy weather as it is to shut him up in over-heated still air of a room. It is as absurd to make a consumptive sit in his damp clothes after a walk in the rain as it is to prevent him taking exercise in the rain. The middle course in all things. Greater exposure for the more vigorous patients, less for the emaciated and weakly. Let us seek gently to excite the metabolism and appetite and lower the fever by open air, but do nothing to depress the bodily functions by exceeding the heat-regulating capacity of the patient and chilling him, or submitting him to discomfort and even misery.

There is no advantage in exposing feeble and ill people to cold wet weather or mist. They are then better in shelters or wards tempered by the radiant heat of a fire, with ventilation enough to keep the air pleasantly cool and moving, with fire enough to keep the relative humidity down, and a pleasant sense of radiant warmth. Hot-water pipes placed under the floor beneath the beds of bed patients,[3] or feet of sitting patients, and open windows will give the right conditions.

The ideal conditions out of doors are sun to promote the feeling of comfort and happiness, a gentle cooling breeze to promote adequate cooling of the skin and stimulate the metabolism of the body, coolness and low-vapour tension of air to promote evaporation of water from, and blood-flow through, the respiratory membrane. In wet, wintry weather in our climate these conditions can be imitated by a fire or hot-water bottle and window ventilation, advantage being taken of every bright day to place the patient out of doors exposed to sky shine, and so far as is comfortable to the radiant energy of the sun, and so far sheltered from wind as to secure for him no more than a gentle cooling breeze.

[1] H. G. Barbour, *Arch. Int. Med.*, 1919, **24**, 624.
[2] Ib., *Proc. Soc. Exper. Biol. and Med.*, 1919, **16**, 136.
[3] Sir H. Gauvain uses electrically heated mattresses at the Treloar Home, Alton.

The greatest care should be taken to prevent overheating the patient by the sun. The sun should be allowed to keep the patient's naked skin comfortably warm, while the breeze cools him, the skin being exposed to the extent which is comfortable to the patient and allowed to become gradually tanned—not severely sunburnt. The eyes of the patient should not be troubled by glare, but agreeable shade should be arranged so that he can read and write in comfort. The patient must not be put in a verandah where he is over-heated by the sun, but must be moved so as to secure cooling breeze plus sun.

The clothes must be arranged so as to secure the maximal cooling power of the skin which is comfortable, the patient being encouraged to secure mitigation of his fever through the cooling power of the atmosphere, but avoiding any uncomfortable feeling of chill, having at his disposal an extra wrap or hot bottle to the feet. He must be gradually trained to endure more exposure to cooling power as his metabolism improves under the treatment. The treatment can be controlled by kata-thermometric measurements of cooling and evaporative power, and black fur and check surface temperature measurements.

As the patient becomes able to take exercise, a climate with sun and cool, dry, moving air, such as that of the winter alpine resorts, will impel him to keep himself warm by exercise, and fill him with the joy of living. Our southern seaside resorts with cliffs which intensify the radiant heat of the sun and provide shelter from inclement wind, afford the proper winter exercise grounds for the weakly. Gradually, as the cure proceeds, the walks can be taken on the downs instead of the shore, climbing and the full strength of the wind coming then into play on the metabolism.

In most countryside sanatoria sheltered level walks can be arranged on the one hand, and open hill climbing on the other hand. Sanatoria should be placed where there is the dryest soil and the most hours of genial, bracing, sunny weather can be obtained. Sanatoria should not be placed in damp, low, positions, or be too closely shut in with trees; neither should they be exposed to the full searching power of the wind. Quick-drying, sandy soil is to be preferred.

Of very great importance is the wise discipline of the daily life of the patients at a sanatorium.[1] The mental and emotional nature of the patient must be regarded, his favourite environment, resources in amusing himself, love of company, &c. Too often are the patients told to walk so far and rest so much, but left to amuse themselves or mope through the day. There should be one to guide them such as a wise schoolmaster trained in the control of physical exercise and amusements, capable of instilling joy of life: one who can map out the patients' day, the walks, gardening, games, &c., control card playing and gambling, and prevent drifting to public-houses; one who will introduce visitors from among the more convalescent patients to such of the bed cases who are not being treated with absolute rest, regulating their visits, and breaking the hours of loneliness and depression, while controlling the hours spent in reading, and introducing other methods of passing the time such as embroidery, making of scrap-books, various

[1] Such as that of Marcus Paterson at Frimley and Gauvain at the Treloar Home.

forms of handwork such as practised in kindergartens. To secure a cheerful mind in the consumptive patient is of first importance.

Admirable results were obtained in the army with cases of relapsing malaria fever by taking them away from hospital environment and slowly restoring their resistance by food, fresh air, and light exercise. So too with cases of vaso-motor instability, and minor poison-gas casualties. Open-air treatment of septic wounds has proved of great benefit.

Berkeley Moynihan says:[1] 'In the treatment of all gunshot wounds where the septic processes are raging, and the temperature varies through several degrees, an immense advantage will accrue from placing the patient out of doors. While in France I developed a great affection for the tented hospitals. There is great movement of air, warmth, and comfort; when a sunny day comes the side of the tent may be lifted and the patient enjoys the advantage of open-air treatment. Wounds which were sullen in healing quickly brightened and became clean when exposed to the air and sun. Even in the winter, and in Leeds and Sheffield, we have some patients out of doors day and night. I saw at the 3rd Northern General Hospital a row of beds in the open occupied by ruddy, happy men with quickly healing wounds. I asked many of the patients how they liked the exposure, and all at once replied that they were quite happy and "wouldn't go inside to sleep if they could help it". I am confident we do not sufficiently take advantage of the immense help which open-air treatment can give to patients suffering from the serious wounds of war. I find that wounds clean more rapidly, patients sleep better, eat better, and feel better when they are kept out of doors night and day. The vagaries of the temperature chart need not influence our decision as to the wisdom of this treatment. Many years ago I learnt at Johns Hopkins Hospital that a high septic temperature was an indication for moving the patient on to a balcony rather than a reason for dreading the effects of exposure.'

After much experience and loss of life from the opposite practice the widely laying open of wounds at the clearing stations at the front came to be the routine practice. To swathe the wounded and send them to the base for operation meant that heat and moisture, confined by the dressing, favoured the development of gas gangrene, checking the outpouring of lymph. The controlled promotion of evaporation from wounds by moving cool air is a subject worthy of the surgeon's attention.

Ellsworthy Huntington[2] has sought to prove by statistical evidence that the deaths from operation in Boston City Hospital are affected by climate. The admission of cool air to the patient is, he says, as important as the proper preparation of the diet.

Warmth is necessary to mitigate shock in conditions where the normal thermo-regulating mechanism is out of action, the tone of the body lost and the blood gravitating into dependent parts. The Inter-Allied Surgical Conference in November, 1917, agreed that in genuine shock warming of the patient is essential in any treatment, and all beds for such patients should be 'heating' beds.

[1] *Brit. M. J.*, March 4, 1916.
[2] *Mod. Hosp.*, 1920, **14**, 10 and 111.

A surgeon at the front wrote to me: 'I think it will be well within the mark to say that during the cold weather the number of patients admitted to the ward for resuscitation treatment is relatively increased. These people are suffering from shock and are extremely cold. It is more difficult generally to get them "warmed" up and to react to treatment than during the warmer weather. Patients who have been "lying out" have almost invariably severely infected wounds, but I do not think this is more the case in cold than in warm weather. I should say, therefore, that in cold weather the shock is increased, and that the vital reaction of the man as judged by his response to measures making him fit to undergo operative treatment is diminished.'

Exposure to cold and damp in the trenches might precipitate an attack of inflammation in those who had some latent septic focus present about them, the inflammation being attributed directly to the exposure when it is really due to the hidden septic focus.

The value of radiant heat for restoring cases collapsed from exposure to cold has received practical demonstration and deserves wide recognition.

Air-Pilots who 'crashed' in seaplanes, and were immersed in the sea supported by their life-belts, to be rescued later by a motor-boat, were often some considerable time before reaching the first-aid room, and in the winter arrived 'chilled to the bone', with teeth chattering and extremities almost blue with cold. They may take as long as four hours to recover warmth when put in bed and surrounded with hot-water bottles, and suffer much, just as one does from pain in fingers chilled by snow.

Electric lamps hung to a cradle, and covered with blankets, were found excellent in promoting quick return of warmth.

Patients in operating theatres should be kept warm by electric heaters, and the theatre kept cool for the surgeon and nurses.

CHAPTER VII

Treatment at High Alpine Altitudes.

At high altitudes paper impregnated with iodide of potassium and starch turns to the deepest blue. This is due not to ozone but to oxides of nitrogen, of which there appears to be three to five times as much at 12,000 feet as, say, in Paris.

The air at the sea contains also more oxides of nitrogen than in land air, particularly in sunny weather, also 2 to 5 mg. of salt with traces of iodine, bromine, &c., per cubic metre of air, all of which may have a slightly provocative effect on the secretion of the respiratory membrane. The sea keeps the air cool and in motion, there is little shelter from wind, cooling power is high, the sandy ground or rock is quickly warmed by the sun, in spite of high relative humidity the evaporative power of the cool, moving air exerted on the skin and respiratory membrane is high.

Sea and high mountain air are almost free from dust and bacteria —these are caught by sea or snow-fields—and while the sea air is relatively free from mist, alpine air has none in winter.

Both sea and high mountain have far more sunlight. Sunlight is not only direct but reflected from sea or mountain snow-fields. In November, December, January, and February the hours of sunshine at St. Moritz, 1,856 metres, are twice as many as in Zurich, 410 metres.

'Endless snow-fields, doing duty as reflectors, with the upper surfaces of endless sheets of lowland fog, innumerable peaks with their faces turned south doing duty as hot plates—such is the provision made for an immense syphon-like circulation in the becalmed winter air.'

Roget says that 32 per cent. of the radiant energy of the sun, as measured by its heating effect, is estimated to be lost at sea level, 21 per cent. at 1,200 metres, 11 per cent. at 3,000, 6 per cent. at 4,810 metres, the height of Mont Blanc.[1]

While the shade temperature keeps more even between night and day at high than at low altitudes, the thermometer may range from −30° C. at midnight to +50° C. exposed to the sun in the mid-day sun. The blackened thermometer *in vacuo* may reach 101° C. in Thibet (midsummer).

It is cold in the shade and hot in the sun; a high cooling acts on the parts of the body in the shade, while the parts exposed to the open are warmed by absorption of radiant energy.

Such readings may be obtained in winter as:

	Shade	Sun
Gornergrat, 3,140 m. high . . .	+14° C.	+47° C.
Diavolezza, 2,986 m. high . . .	+6	+59½

(Roget).

[1] F. F. Roget, *Altitude and Health*, London, 1919.

The bare ground is warmed by the sun on the mountains just as the sand is on the sea-shore. A sea bath and then to lie in the sun on the heated ground is most grateful to the senses.

You may enjoy, says Roget, a hot cup of coffee while lounging in the sun, but if by any chance the shadow of your body falls upon the cup, your drink may be frozen. These quick alternations are as conducive to vigorous life as a monotonous climate is depressing.

The possible contrasts of climate are prodigious in the Alps. Roget cites the valley of Findelen, above Zermatt, which runs west and east; on the slope facing north the vegetation is that of the Siberian tundra, on the other, cereals ripen at an altitude of 2,100 metres, and the vegetation is that of the arid barren tracts on the Mediterranean border. While a well-aimed stone can traverse the geographical distance, the climatic distance is from Iceland to the rocks of Gibraltar.

The invigorating Alpine air is at times modified by the foehn wind, the result of air currents rising vertically from the plain of the Po, and spreading far above the Alps and dropping thence perpendicularly down the slopes. It may eat through 3 feet of snow in less than 24 hours, and has a great effect on the vine and ripening of fruit amid the Pennine Alps. It has a most depressing action on human beings, muscular effort is irksome, and people feel limp and ill at ease. (Roget.)

At an altitude of 2,000 metres, owing to the lowering of temperature, there is half the moisture in the air as in the lowlands, at 4,000 metres one quarter, at 6,500 metres one-ninth. Hence the transparency and intense chemical action of the sun.

Above 5,000 feet in the Alpine winter the skin feels harsh to the touch from drying, lips have a tendency to chap, throat and eyes may tickle from the drying effect of the air, hair gets hard and electrical, moist eczema and 'colds' dry up. The skin thickens and the hair grows in length and thickness on exposed parts.

Mountain air is rarified, has less oxygen by weight, and greater evaporative power on the respiratory membrane as it is cold and dry, and a larger volume has to be breathed to secure the weight of oxygen required by the body. The lungs are better ventilated, and more blood and secretion flow through the respiratory membrane. The following figures show the partial pressure of oxygen at some well-known places:

	Partial pressure of oxygen	Altitude
Monte Rosa	92	15,000 feet
Pike's Peak	97	14,000
Teneriffe	105	12,000
Col d'Olen	111	10,000
Cañadas	126	7,000 (Barcroft)

The number of blood corpuscles and percentage of haemoglobin is increased to counterbalance the fall in oxygen partial pressure, and as a result of acclimatization to the cold sunny climate. High altitude regenerates the blood as the old-fashioned habit of blood letting. At 1,800 metres there may be an increase in a fortnight of 20 per cent. in corpuscles, and 7 per cent. in haemoglobin, in a month 23 per cent. and 15 per cent. respectively. This effect is shown not only in man but domestic animals.

Rabbits taken up to 2,900 metres and set to breed, after seven years were somewhat smaller, with ears not so large, and coat lighter in colour and of extraordinary thickness, an approximation to the Arctic type. There were 70 mg. of iron in the blood against 40 for the rabbits of the plains. So with sheep and cattle taken to high levels for the summer, the oxygen carrying power of the blood is enriched. Quite a low altitude, e. g. 2,000–3,000 ft., increases the haemoglobin and lowers the partial pressure of CO_2 in the alveolar air.

Haldane, Yandell Henderson, Douglas, and Schneider in the Pike's Peak Expedition, found (by the carbon monoxide method) a considerably higher pressure of oxygen in the arterial blood than in the alveolar air after two or three days' acclimatization. On breathing oxygen-enriched air this difference almost disappeared. They explain this as due to active secretion of oxygen into the blood by the alveolar epithelium, just as in the case of the swim bladder of fishes oxygen is known to be secreted, a secretion under the influence of secretory nerves, and provoked by pilocarpine. The theory that the lung has power to secrete oxygen has been disproved by Barcroft, who, spending five days in a chamber at low oxygen pressure, found the oxygen saturation of his blood, taken from the radial artery, corresponded to the partial pressure in the alveolar air of his lungs.[1]

The mountaineers have nearly everywhere established their superiority as fighting races. The healthiness of life at high altitudes is illustrated by the hardy physique and capacity for toil of the men, and also by the absence of diseases which affect the dwellers in the plains. It is of interest to consider the manner of life and healthiness of the monks of the St. Bernard hospice described by Roget. They rise at 5 in summer, 5.30 in winter, and occupy themselves in religious exercises and church offices till a breakfast of coffee, milk, bread, and cheese. From 8 to noon they study, then dine, getting meat five times a week, fish in Lent. Physical exercise follows the short dinner time, with ski on the hill-side whenever the weather allows. Study follows from 2 till 6.30 with church from 4 to 5. At 6.30 supper is taken, with meat five times a week, followed by recreation, a singing lesson, and evening prayer, and bed at 9.30. Sleep is interrupted by midnight divine offices not infrequently. Once a week the monks are led out on a hardening trip among the hills, supplied with plenty of food and spare clothing, such outings sometimes implying great hardship. The exceptionally robust monks do not thrive so well on the life as those with a disposition towards anaemia and nerves.

After fifteen or twenty years loss of vigour, chronic indigestion, and rheumatism affect the muscularly and constitutionally powerful members of the congregation. These are then transferred to active work in parishes extended over much hill and dale, and their plenitude of outdoor life sets them right again in a few years.

The brethren are free from infective and eruptive diseases except when an infectious disease is brought by a wayfarer to the hospice.

Two monks came infected with pulmonary tuberculosis, and spent the first eight, the second thirteen, years at the hospice before dying. They caused no infection.

In the lofty table-lands of Anahuac, the Cordilleras, Abyssinia,

[1] *Proc. Physiol. Soc.*, Feb. 21, 1920.

Thibet, not contaminated by visitors, in spite often of a wretched standard of living and bad sanitation, phthisis is said to be unknown,[1] and pathogenic organisms are almost absent.

At altitudes of 5,000 to 7,000 feet in U.S.A., lung diseases are rather less than half the proportion experienced on the flat lands. In the high table-lands in Switzerland, before their penetration by visitors, lung disease was practically unknown.[1]

'The alpine sanatoria offer open-air galleries with light pouring down from the vast open sky, and flooded with air. A beautiful world of snowy peaks and hazy valleys spread out beyond. Windowless bedrooms with curtains to keep out snow. With a cloth round their loins the children roll about in the dry, sunny, powdery snow. Practically naked, with skin as black as old oak, they snowball one another, skate, toboggan, fly down the slopes on ski.

Send the children early, says Roget: with adults the disease is hard to dislodge, they have ingrained habits of life, it is difficult for them to give up the frivolous wear and tear of self-indulgence, wakeful pleasures, and false excitements, and abandon themselves to the healing powers of nature. Adults neutralize the good effects of pure air, keen frost, and all-day sun by crowding into central-heated hotels and indulging in luxury and excitements. According to E. Morland[2] the Latin races do not show a healthy reaction to mountain air. For cases of bronchitis a warmer and moister air are desirable. Cases with incompetent hearts, kidneys, or digestive organs are, he says, unsuitable for high altitudes. Alpine climate is almost a specific for asthma in children, the asthma ceasing so long as they remain in this climate.

Surgical tuberculosis responds best to Alpine air and sunlight. While pain is prevented, open tubercular sores dry up and heal when exposed to the sun and air, the diseased tissue crumbles off while a new cell activity sets in. The body as a whole must be carefully protected from over insolation while the patient remains in bed. Out of doors, playing nude in the snows, no such protection is required.

[1] F. F. Roget, *Altitude and Health*, London, 1919, p. 45; H. C. Lombard, *Traité de Climatologie médicale*, Paris, 1877.

[2] *J. State Med.*, 1920, **28**, 129.

CHAPTER VIII

Concerning Clothes.

The skin, with the hair and the subcutaneous fat, forms the natural garment of the body. The hair shelters, in man particularly, the brain and the orifices of the body, nose, mouth, anus, and the genitalia: the hair in the armpits protects the thorax when the arms are raised. The hair protects the brain not only from the cooling power of the atmosphere but also from the radiant energy of the sun. The long hair of a wild woman can protect the infant at her breast; it hangs as a garment round the crouching wild human.

The low thermal conductivity of the skin, depending on its dryness, is diminished in warm atmospheres by turgescence with blood and lymph; the sebum preserves the skin from wetting.

A thick layer of adipose tissue extends the heat-regulating power enormously, as is seen in the case of the Cetaceae which inhabit the Arctic seas. Their cutaneous fat forms a layer impenetrable to water and of very low thermal conductivity, while their enormous muscular mass maintains the production of heat. Similarly channel swimmers require a thick layer of subcutaneous fat. Man amplifies his powers of physical regulation by means of clothes and artificial heat, and thereby can enormously husband his resources and save food. By huddling in the close atmosphere of a one-room tenement the poor can save food. They cannot afford a healthy open-air life. Good wages, then, are the basis of good hygiene.

Clothes protect us from the rubbing and tearing, the wetting and corroding action of the world, the bites of insects, from cold and radiant heat. They afford means of ornament and sexual attraction, the decent privacy of sex, the notation of rank, occupation, and culture, and the means of imposture and exhibition of vanity and arrogance. 'Man's earthly interests are all hooked and buttoned together and held by clothes', says Carlyle.[1] 'Clothes, too, which began in foolishest love of ornament, what have they not become? Increased security and pleasurable heat soon followed, but what of these? Shame, divine shame, arose there mysteriously under clothes . . . a mystic grove-encircled shrine for the Holy in man. Clothes gave us individuality, distinctions, social polity; clothes have made men of us, they are threatening to makes clothes screens of us.'

Historical evidence shows that woollen fabrics were used in the Bronze Age—they were found in a grave in Jutland. Woollens form the principle fabrics found at Pompeii. Camel hair has been used for many centuries. Silk was used in China 4,500 years ago, flax in the neolithic age, and linen is mentioned in the book of Genesis. Cotton was used in India more than 2,000 years ago.

[1] *Sartor Resartus.*

Incredible as it may seem to the well-clad and housed that man should endure nakedness in winter climate worse than ours, yet Darwin describes this of the natives of Terra del Fuego—the woman with babe at her breast, both naked, and the sleet falling and melting on them—the man with at most an otter skin, the size of a handkerchief, worn on the back and laced across the chest and shifted to the side struck by the icy wind. Stunted in growth, with faces made hideous with white paint, they sleep on the ground curled up in forms like hares. Feeding on shell-fish, eggs of sea-fowl, putrid dead whale stored against times of famine, food gained with unremitting toil, they not only do not decrease in number but commit infanticide to keep the population down to the food supply. 'Nature by making habit omnipotent and its effects hereditary has fitted the Fuegians to the climate and productions of his miserable country.'

In contrast with the naked Fuegians, I picture a curate who visited the laboratory on a mild winter day, and complained that he always felt cold. He wore a thick llama wool vest, a thick woollen shirt, a wool-lined waistcoat, a cardigan jacket with long sleeves, a tweed suit, and a wool-lined motor overcoat. Why should this guardian of men's souls thus induce the perfect heat-regulating mechanism of his body to atrophy from disuse? Nursery training had instilled into him the fear of cold, draught, and wet feet. And yet the channel swimmer Burgess, coated with grease like the Fuegians, stood exposure to sea-water for some twenty-three hours, and the cooling power of water measured by the kata thermometer is 14 times greater than air.

On to his ordinary clothing an officer going on night-watch (North Sea, winter) put a cardigan waistcoat, a life-saving waistcoat, a thick muffler, a coat, a second pair of socks, and then a pair of thick woolly sea-boot socks, and lastly his sea-boots. The curate, then, faced the mild winter weather of London in clothes fully as warm as those worn by the officer who spent the night-watch on the bridge in the North Sea. What a range of adaptability of the body habit is there between the curate, the channel swimmer, and the Fuegian. So hardy are the Highland soldiers that, wet with snow in wintry weather, they would take off their kilts to dry them on a brazier, and think nothing of standing in the trenches without them.

A civilized man when naked finds exposure to still air at 35° to 37° C. bearable; 25° to 30° pleasant; 15° cold; while 10°–12° in a few minutes gives him an intensely cold feeling.

For sedentary occupation he secures a temperature approximating to 33° around his skin by means of clothes and artificial heating; constantly trying to remove himself from the influence of cold which would increase heat production, his daily life is spent practically under the influence of a tropical climate; only about 20 per cent. of his body-surface is exposed to the air. An artist's model can stand exposure to 14° C. for several hours without shivering and there may be 10° C. difference between the skin of the waist and ankle.[1]

Rubner says the clothing needed for a temperature of 25° are a light wool undergarment, and a coat of linen or silk; at 30° sweating cannot be prevented by any modification of clothing; at 15° summer clothing is required; at 8°–15° summer clothing plus

[1] Benedict, *Proc. Nat. Acad. Sc.*, 1919, **5**, 218.

a light overcoat; at 8° to −4° winter clothing, thick undergarments and overcoat; at and below −4° winter clothing and a fur overcoat. Rubner takes no account of wind in this statement. The clothing needs out of doors are settled by cooling power, not temperature.

Custom and fashion impose on the people clothing which is either unsuitable in character or too much. The great error is lack of ventilation. Too heavy clothing is less of an evil than badly ventilated clothing, because the latter provokes excessive sweating and leaves the skin needlessly long in active state. Clothing should allow great adaptability of body to change of temperature. It should not provoke sweating in the resting-subject in still air at too low a temperature, e.g. at 27° instead of 30°.

People are generally dressed unsuitably for the heat of summer. Only the rich can adapt their clothing, and the English climate is too changeable to permit much adaptation for those who go to business, and are away from home all day. In hot weather the needs are to reduce clothing and food, in particular meat, to bathe, and secure fan ventilation.

The opinion of an observant Scot practitioner is here worth quoting. He writes to me: 'The longer I work among people, the more I find cases of individuals who habitually go with little clothing and still show the greatest immunity to those diseases commonly attributed to the influence of cold. I am becoming more and more convinced that a great deal more harm comes from overheating the body than is ever due to the influence of natural cold.' Children in elementary schools are reported as wearing as many as 13 different articles of clothing.[1]

The Commissioner of the Gilbert and Ellice Islands reported that the introduction of clothing, a cotton smock for women, and cotton trousers and shirt for men, had had a bad effect on the natives, reducing the endurance of the skin and causing chills. A kilt of leaves and inunction of the skin with coco-nut oil was the native custom. The clothing of native races is said at once to increase the incidence of pulmonary tuberculosis. The spread of the white man's infectious diseases, syphilis, tuberculosis, &c., among non-immunized people is not, however, a question of clothing. The disability produced by clothes in limiting heat-loss in tropical climates has not prevented millions of negroes taking to them or prevented the negro rapidly increasing in proportion to the whites in America and the West Indies.

Clothes act as an elastic cushion, their compressibility and elasticity being important properties in affording protection and comfort. The advantage of doubling the layer is strikingly seen in the case of socks. Double socks rub against each other and protect the feet and wear better. They afford a better cushion to stand on and are of course much warmer.

The clothes need to be as light as possible, permeable to air, allowing free evaporation, becoming to the least degree wet with sweat, not clinging when wet to the skin.

A cellular structure produced by weaving, whether of wool, cotton, or linen, secures the conditions which give the material its warmth, compressibility, porosity, permeability, and evaporative power.

[1] Chesser, *Proc. Inst. Hyg.*, Jan. 28, 1920.

Smooth, closely woven materials, particularly starched and glazed, are unsuitable, for permeability to air is bad and the humidity is greater under such, the sweating more provoked, and muscular activity checked. Coarse clothing materials are, then, better than fine.

Fabrics which prevent free evaporation—non-porous, impermeable materials—have strictly a limited use (1) as outer wind-proof garments for people exposed to great cooling powers, Arctic travellers, aeroplanists, sailors; (2) for preventing the penetration of the tropical sun, then best arranged as a flowing, flapping garment with slits allowing ample ventilation; (3) as a waterproof to keep off rain from a man who has to go to and fro in town. On rainy days impermeable waterproofs are unsuitable for exercise; woollens should then be worn and changed on coming in, or dried off by further exercise when the rain is over. Wet garments cannot chill the body so long as it is kept warm by exercise. Weather-proofed garments have the thread permeated with proofing material and are as freely ventilated as unproofed material.

The effect of a hot fomentation, covered by oilskin, is to soften the skin by imbibition of water. Just the opposite is required, the hardening effect of cool, drying wind. An outer garment of close-meshed fabric when wet prevents evaporation of sweat and increases sweating. Clothing should not over-heat the body and check muscular activity—the very first essential for health.

Over-warm clothing provokes an excessive evaporation from the skin and keeps it in active state unnecessarily long. The health of all is depressed by over-clothing, while it is only the underfed who suffer from scanty clothing. The winter and summer pelts of the warm-blooded domestic animals, horse and cow, indicate clearly the small degree of extra clothing which is required to face an open-air life. Thick undervests and heavy winter overcoats and furs are the necessity of fishermen, sailors, Arctic travellers, soldiers in the trenches, who have to face the cooling power of the winter wind. They are worn by those who destroy their vigour by a sedentary indoor life and a stupid fear of exposure.

While Merino sheep introduced into the Congo, Sudan, and Tripoli lose their wool and have a pelt like a greyhound, camels and dromedaries taken from Africa to Thibet put on a hairy coat. 'The horse', says Carlyle, 'is his own sempster, and weaver, and spinner; nay, his own bootmaker, jeweller, and man-milliner. . . . A perennial rainproof of court-suit on his body wherein warmth and easiness of fit have reached perfection. . . . . While I, good heaven, have thatched myself over with the dead fleeces of sheep, the bark of vegetables, the entrails of worms, the hides of oxen or seals, the felt of furred beasts. . . . A moving rag screen overheaped with rags and tatters raked from the charnel house of Nature.'

Fur or feathers have enormous heat-retaining powers coupled with great lightness and pliability. The clothes require to be fashioned to attain these ends with easy adjustability to climatic changes, and contrived so as to allow man to work with ease. They are fashioned largely to satisfy the vanity of the idle, to impose on others, and to maintain a false system of sexual selection, selection which should be based on the perfection of body and character.

The heat-retaining power of feathers, fur, clothes, depends not on

the low conductive power of the material but on the air entangled in the pores of the material and immobilized therein, and between the layers of material. Air immobilized in the pores of feathers, e.g. swan's down, is a non-conductor of heat. The difference of density between the warm air in and cold air outside the fur causes convection currents, but the resistance is enormous in the capillary channels formed by the hair. The feathered or furry animal must so hold itself to the wind that the fur or feathers are not blown up or disarranged.

Dry, still air at 0° C. is comfortable; moist air at 0° C. unpleasantly cold; water at 0° C. intensely cold, quickly numbing sensation; a bar of iron at 0° C. instantly painful to touch. The thermal conductivity of iron is 120 times as great as that of water, and that of water is at least 240 times as great as that of air, and its heat capacity more than 3,000 times that of air. No iron-shod boots or iron stirrups should be worn by Arctic travellers. They require to be clothed in a light waterproof and *wind-proof* flexible outer cover, and a thick layer of air-holding fluffy material beneath. The great trouble of Arctic travellers is the moisture taken up by their sleeping-bags by night which freezes by day. The problem is how to ventilate the sleeping-bag by night so as to let moisture escape and yet keep warm. Wolferine is a fur upon which the moisture in the air is said not to freeze. It is used as a head-dress by Eskimo.

If the thermal conductivity of air be taken as 1, that of wool fibre is 6, silk 19, cotton and linen 30. These figures refer to fibre free from air, not clothing material. The practical thermal conductivity of a clothing material is based on its specific weight—in which measurement the air in its pores is included. Rubner[1] gives the following:

The smallest specific weight of flannels is . . .	0·09-0·15
The average „ „ „ „ . . .	0·2 -0·3
The highest for smooth materials	0·4 -0·7
The average for furs	0·05

The hairy part of fur is 98 per cent. air and 2 per cent. solid substance, flannel 90 per cent. air, overcoating 75-80 per cent. air, smooth materials at least 50 per cent. air, but starching, glazing, loading may make a material airless.

	Thickness	Thermal conductivity
Cotton stocking	0·745 mm.	0·0039
Upper leather of shoe	1·0	0·00116
Woollens	2·7	0·00025
„	3·21	0·000195
Leather sole (it contained 42 per cent. of air)	11·0	0·000105

Leather has air and grease in it, the oil protecting against the permeation of water.

The permeability of a garment to wind stands in relation to its pore volume and specific weight. Smooth, thick stuffs are least permeable, and loose woven stuffs most. The permeability has been measured by the time in seconds it takes to force 1 c.c. of air through 1 sq. cm. surface 1 cm. thick with a water pressure of 0·42 mm. Rubner gives these figures:

Dressed linen	171 sec.
Linen washed free of dressing	17·2 „
Thick cotton	76 „
Knitted stocking	15·7 „

[1] *Lehrbuch der Hygiene*, Leipzig, 1907, 8th edit.

The smooth cotton linings of woollen cloth garments have a profound effect on permeability. Thus :

109·3 sq. cm. of a lining let 5 litres of air through under a given pressure in 266 sec.

The same lining with winter woollen cloth in 282″.

Lining plus cloth plus lining in 590″.

Smooth linen, cotton, and silk stuffs vary in thickness from 0·16 to 0·4 mm. ; knitted, 0·6–1·2 mm. : flannel, 2–3 mm. ; overcoating, 6–7 mm. ; fur, 12–40 mm.

The average thickness of clothing should be, according to Rubner, for such as can have wardrobes adapted to all weathers :

	Hot summer	Summer	Spring, Autumn	Winter	Very cold winter
Thickness . . .	1·76 mm.	3·36	5·92	12·62	26
The per cent. of fibre to total thickness	17	23	26	13	6

The average clothing of the whole body is, he says, 8·6 mm. thick, weighs per 1 c.c. 0·42 grm., and has a specific weight of 0·27, consisting of 146 parts solid and 854 parts air. The summer clothing weighs 2·5–3 kg. ; the winter 6–7 kg. In winter a man carries one-tenth of his weight in clothes, while a dog weighing 4–5 kg. has only 70 grm. of hair to carry in his winter pelt, less than 2 per cent. of his weight.

While cotton fibre is 92 per cent. cell wall and 8 per cent. lumen, linen fibre 98·2 per cent. cell wall and 1·8 per cent. lumen, wool fibre is solid. Cotton and linen fibres are smooth, but the hair (wool) fibre covered with imbrications comparable to those on a fir-cone. It is these imbrications and the elastic projecting nature of the hair fibres which give the tickling, irritating effect to the skin, so acutely felt by children who for the first time put on a fluffy woollen garment next to the skin. The smooth character of cellular cotton or linen garments and the greater heat conductivity of the vegetable fibre offer advantages for tropical use, but such garments cling more when wet with sweat.

For tropical heat an open-meshed cellular cotton or linen garment is best, the skin being tanned. To prevent sunburn in the untanned a garment must be worn close enough to prevent penetration of the sun's rays. It must be loose enough to be well-ventilated, a single flapping sun-proof white robe.

The advantages of woollens are these. The elasticity of the hair keeps the garment off the skin and secures an air-layer beneath, facilitating evaporation and preventing the clinging wetness of a smooth garment. They possess great compressibility and softness and are less destroyed by sweat. On the other hand, they thicken and shrink on boiling, the porosity becoming thereby diminished. Methods of preparation and weaving of the wool can minimize this. For hot weather, woollens with their lower heat-conductivity and greater thickness are too warm.

Rubner distinguishes the primary properties of the fibres from which the clothing materials are made from the secondary properties due to the mode of weaving. The primary properties are elasticity, power to absorb water vapour, ease with which wetted, amount of swelling when wetted, resistance to destruction in cleaning and washing.

The secondary properties, due to weaving, are content of air, permeability to wind, heat-conductivity, water-holding capacity, and evaporative power, radiant heat emissive power, compressibility.

The claimants for systems of clothing all wool, linen, &c., have based their claims on the nature of the material—on the fibre—while the properties of a garment depend almost wholly not on the fibre, but on the manner of its weaving, and the same results can be secured with wool, cotton, or linen by weaving out of each a material with the same porosity and air-holding power, permeability to moving air, and water-evaporating power.

Jäger insisted on the sedative power of wool on the life process. He upheld the reduction of metabolism as a means of preserving life, as if the body burnt out like a candle—a preposterous doctrine.

Wool next to the skin is warmer because it is thicker than a smooth fine cotton or linen garment. A fine linen handkerchief lets ten times more heat through than a flannel shirt because of its thinness. Weave the linen equally thick and porous (air-holding) and it is as warm. Thus dry cotton flannelette is no less warm than flannel. The flannel is the warmer when wet, but the clothes of the citizen are rarely wet.

The protective power of clothes. The effect of shaving off the long-haired fur coat of a dog is shown by the following (Rubner):

	Heat loss Calories per kg.	
Temperature of chamber	Normal	Shaved
7·6° C.	86·4	
15·0	63·0	82·3
20·0	55·9	
25·0	54·0	61·2
30·0	56·2	52·0
35·0	68·5	

Thus the dog's power of regulating its temperature by controlling heat loss in a room between 20° and 30° C. was lost after shaving. A guinea-pig's heat loss increased 36 per cent. in an enclosure after shaving. A pigeon's metabolism was doubled after removing its feathers. Varnishing an animal has the same effect as shaving, it greatly increasing the loss of heat. A rabbit shaved and varnished had its metabolism raised 140 per cent. at a room temperature of 15° and 20°. Exposure to wind would of course greatly increase these heat losses.

Varnished or shaved animals, just as those exposed to excessive cold for a prolonged time or too often, die of inanition and collapse. They respond at first by increased metabolism and then become exhausted.

Lefèvre found a turkey after being plucked lost heat three times more quickly in a bath at 12° C. A naked pigling lost heat in the bath three times more quickly than a dog and four times more quickly than a rabbit. The air entangled in the feathers or fur has a very great protective power. Not only is the loss of heat by convection increased greatly by shaving, but the emissivity of radiant heat. Using a thermopile I found the emissivity of radiant heat of the shaved skin of a guinea-pig compared with the unshaved was such as to give a 25 per cent. increase of the galvanometric deflection.

From Lefèvre's measurements I calculate the effects of light clothing on man as follows:

Weight 65 kg : surface 19,000 sq. cm.
Temp. 9° : wind 1·2 m. per sec.
Sitting nude 134 kilo-calories per hour = total loss ;
Less 24 calories for loss from evaporation = 110 cal. per hour ;
= 1·6 millicalories per sec. per sq. cm.
Sitting clothed lightly total loss = 98 cal.
Less 24 calories for loss from evaporation = 74 cal per hour per 19,000 sq. cm.
= 1·0 millicalories per sec. per sq. cm.

$$\frac{\text{Loss of heat naked}}{\text{Loss of heat clothed}} = \frac{1.6}{1} = 1.6.$$

Temperature 4° C. : wind 4 m. per sec.
Naked ; total loss = 313 kilo-calories per hour ;
Less 24 calories = 289 calories per hour per 19,000 sq. cm.
= 4·2 millicalories per sec. per sq. cm.
Clothed lightly ; total loss = 170 cal. per hour per 19,000 sq. cm.
Less 24 calories = 146 „ „ „ „ „ „ „
= 2·1 millicalories per sec. per sq. cm.

$$\frac{\text{Loss of heat naked}}{\text{Loss of heat clothed}} = \frac{4.2}{2.1} = 2.$$

The heat loss of the naked man at 4° C. and 4 m. per sec. wind is then four times that when clothed at 9° C. and wind 1·2 m. per sec.

Lefèvre also measured the loss of heat at 4·5° C. and a wind of two metres per sec. The loss in kilo-calories per kg. per hour was :

Naked man	Pigling	Dog	Clothed man	Rabbit
8·6	8·0	4·7	4·3	3·8

The clothes halve the loss and make the man's loss between that of the furred dog and the rabbit, the loss of the naked pigling closely approximating to that of the naked man.

Birds in the Antarctic at, say, −35° C., have a body temperature of 43·5° C. or 79° above that of the air, showing the protective power of a well-greased unruffled coat of feathers.

The effect on the metabolism of removing overcoats, &c., while sitting out on cold winter days is shown by the following observations which Miss Hargood Ash and I carried out by the Douglas-bag method :

Subject L.H. Sitting in open shelter. Snowing :

Temperature		Cooling power		Cheek
Dry bulb	Wet bulb	Dry kata	Wet kata	temperature
1·0° C.	0·7° C.	23·5	44	20° C.
Heat loss with overcoat, comforter, gloves, and rug		1·15 millicalories per sq. cm. per sec.		
Heat loss without overcoat, &c.		1·87	„	„

Increase in heat loss 62·6 per cent.

Subject D.H.A. Sitting in open shelter. Slight breeze, snowing at times :

Dry bulb	Wet bulb	Dry kata	Wet kata	Cheek temperature
2·5° C.	below 0° C.	19·7	33·8	20·0° C.
Heat loss with thick overcoat and muffler		1·48		
Heat loss without overcoat and thin clothes, shivering		2·61		

Increase in heat loss 76·3 per cent.

Darwin in the Voyage of the *Beagle* relates the following story: A party was crossing the Cordillera in the month of May; a furious gale of wind arose so that the men could hardly cling on their mules, and stones were flying along the ground. The day was cloudless and not a speck of snow fell, but the temperature was low. It is probable that the thermometer would not have stood very many degrees below the freezing-point, but the effect on their bodies, ill-protected by clothing, must have been in proportion to the rapidity of the current of cold air; the gale lasted for more than a day. The guide's brother tried to return and perished, two men lost their fingers and toes. Out of 200 mules and 30 cows, only 14 mules escaped alive. Here is an illustration of the cooling power of a gale on fur-coated animals. They must and do seek shelter, and man to face such wind has to protect himself with unruffled wind-proof materials.

The design of aeroplanist or Arctic clothing should be based on an outer layer of wind-proof material, e.g. waterproof cloth, the lighter the better so long as it is wind-proof, and inner layers of soft, fluffy woollens entangling plenty of air, garments joining each other in such a way as to let no wind in at cuff, waist, or ankle. A tunic with helmet all in one piece, gloves pulling up over sleeve cuffs and confined to them by straps, trousers coming well up above the tunic, and the two confined by a belt, long boots made of supple hide coming well up over trouser legs, with soft woollen socks inside.

Rubner studied the respiratory metabolism of a man resting in a chamber at 11°–12° C. The dry kata cooling power in still air at this temperature is 7.

The effect of clothes was as follows:

	CO_2 in grm. per hour	H_2O in grm. per hour	
Summer clothes	28·4	58	Cold, occasional shivering.
Do. and winter overcoat .	26·9	50	Chilly part of time.
Do. and fur coat . .	23·6	63	Comfortably warm.

When comfortable, chemical regulation was eliminated, and metabolism depressed to the low level. This man must have been unused to open air to shiver when sitting lightly clothed in a room at 53° F.

Rubner says the naked parts of the body in an enclosure at 14° C. have an average surface temperature of 29°, the clothes 21° C. The difference is larger with thicker clothing. The surface temperature of the hair or beard is like that of the clothes. The skin temperature in different parts of the clothed body may vary by as much as 6° C., being highest at the waist.[1]

In a room the following were found:

Air temperature	Difference between air and naked parts	Difference between surface of clothes and air
10° C.	19° C.	9·3° C.
12	14·2	6·0
17·5	12·5	5·4
25·6	5·6	1·0

[1] Benedict, *Proc. Nat. Acad. Sc.*, 1919, **5**, 218.

The clothed skin temperature was warmer than the naked skin at room temperature:

10° C.	by	3·2° C.
15	,,	2·4
17·5	,,	1·4
25·6	,,	1·0

Rubner also gives the following readings:

	Room temperature 10°	Room temperature 26°
Temperature of surface of coat .	21·8° C.	28·0° C.
,, between coat and waistcoat .	23·1	28·8
,, waistcoat and linen shirt .	24·4	29·3
,, linen shirt and wool vest .	25·2	29·6
,, wool vest and skin . .	32·7	32·1

The variations only affected the outer layers. Out of doors exposed to wind in the winter the differences are, I find, much greater:

	Atmosphere.		Between shirt and skin of chest		
	Dry bulb	Wet bulb	Dry bulb	Wet bulb	
Jan. 11	0·5° C.	0° C.	18° C.	17° C.	Bicycling.
	10·5	—	29	27½	Indoors, sitting by fire.
Jan. 13	−3·0	−0·5	17	8	Bicycling downhill.
Jan. 15	4·5	4·0	22	20½	Running, warm and moist skin.
	12	10·5	27	24	Indoors, by fire.
Jan. 16	−0·5	−0·5	14	8	Bicycling.
Jan. 19	−0·5	−0·25	20	16	,, uphill.
			14	11	,, downhill.
Feb. 22	6	5·5	28·5	26	Walking, warm.
Jan. 12	1·75	1·5	19	17	Bicycling.
Jan. 3	4	3·5	23	23	Bicycling, warm with exercise.

The effect of clothes in increasing evaporation in the case of indoor workers was recorded by Rubner on a man resting in light summer clothing in the moderately humid air of the calorimeter. The experiment lasted 6 hours:

Temperature of air	Heat production calories per hour	Loss of heat in calories per hour by evaporation	Loss of heat in calories per hour by convection and radiation
15°C.	84·8	21·8	63
20·4	78·6	32·4	46·2
23·8	73·4	43·6	29·8
25·4	82·9	45·2	37·5
28·9	86·6	63·0	23·5

These figures only apply to chamber conditions. A strong wind would keep the evaporative loss down in spite of the temperature rising.

The sweat evaporated by a man resting at 30° C. in still air was 25 grm. per hour naked, 85 grm. clothed. The evaporation of 60 extra grm. of water equals 35 calories. That is, an additional 1,440 grm. of water were evaporated, equal to a loss of 840 calories per diem, owing to the clothing in the tropical conditions of warm, still air.

The protection from insolation. The power of clothes to absorb sunlight depends on colour:

If white	absorbs	100
light yellow	,,	102
,, green	,,	152
dark yellow	,,	140
,, green	,,	161
red	,,	168
brown	,,	198
black	,,	208

(Pettenkofer.)

The absorptive power for dark heat rays is about the same for white and black. Open-meshed clothing materials, particularly white, are permeable to sun rays. Close-woven, loose, impermeable garments,[1] with pith helmets, are needed by untanned whites who go out in the tropical sun. It is far better to become tanned and wear the thinnest cotton material and so avoid prickly heat. While tanning the skin must be protected whenever it becomes tender and red. To labour in the tropics the white man strips to the waist and, tanned and immune to sunburn, his evaporative loss is untrammelled. Clothes for the tropics should have great reflective power, little absorptive power, allow little penetration of the sun's rays, and good ventilation—a flapping, loose robe, such as is worn by the Arabs, provides the requirements, or loose shorts widely open at the knees, and a shirt short in the arms and with slits at the sides and low-cut collar-band. Free ventilation is the great thing. A loose shirt is far better than a close-fitting tunic. Shorts require flaps to turn down at night to prevent mosquito bites. The axilla should be shaved. The sun helmet of the lightest pattern should be fitted with a Simpson net hood (non-inflammable) which allows smoking and prevents insects biting (A. Balfour).

The native Indian cattle and Arab white horses have a white or light-coloured fur to reflect and a pigmented skin beneath to prevent penetration of sunlight. It has been suggested that red linings should be added to white garments to absorb the sun's rays. (Maude.) An 'aertex' cellular cotton undergarment dyed red is advertised for tropical wear, 'so that only the red and orange rays are allowed to act on the body'. The efficacy of such depends not on the red colour but on whether the sun can penetrate through the pores of the garment. A single white garment is far better, woven so as to be impenetrable.

Phalen[2] compared 500 troops marching in the Philippines dressed in white with 500 wearing also a red undergarment. The latter added materially to the burden of heat upon the men.

The heavily loaded cheap calico sold to the natives of India serves the purpose of keeping out the sun's rays.

Some tests on sun helmets carried out by Major Wanhill at Mhow in India showed that the temperature inside the helmets is the same whether red or white lining is used, that reflected sun-heat from wall or ground helps to raise the temperature within the helmet, that wind is the great cooling agent, and that ventilation of the helmet must be adequate. In shelter and sun the inside temperature of the helmet

[1] A single layer of calico is enough.

[2] *Philippine J. Sc.*, 1910, B. **5**, 525.

rose to 110° F., while in a wind and sun it was 102½°–105°. (The dry bulb was 86° F.)[1]

A layer of black hair 1 mm. thick lets through less sun rays than a similar layer of blond hair, but there is no difference between layers 3 mm. thick. The superficial curly hair of a negro, just as black fur, shows a surface temperature up to 60° C. in the sun. It allows free ventilation while limiting penetration. A layer of white skin 1 mm. thick and free from fat lets through twice as much radiant energy as negro skin, using a Nernst lamp as the source and a thermo-electric method as the measure of energy.[2] In the case of white and black flannel the relative permeability of the different rays of sunlight was as follows:

	White flannel	Black flannel
ultra red	0.0118	0.0115
dark red	0.0259	0.0241
red	0.0183	0.00127
green	0.0229	0.0012
blue	0.0104	0.0

A double-roofed tent is much cooler than a single roof; the inner roof may be black. The air between the two roofs is a bad conductor, and convection will prevent the temperature rising much if free ventilation is allowed. All bungalow, carriage, and tent roofs in the tropics require, then, to be double, and the air space between the roofs freely opening so that there is an unimpeded escape for the air heated by the outer roof. So too with ship awnings.

H. E. Roaf investigated in Egypt the effect of white and dark sun-screens on a black-bulbed thermometer placed in a test-tube. A piece of dark blue sail-cloth was placed below, 7 feet from the ground, to cut off upward radiation; 16 cm. above this was placed the screen, and 6 cm. below it the thermometer:

White crepon reduced the temperature on the average . . .	21.7° F.
Dark blue ,, ,, ,, ,, ,, . . .	29.8
White sail cloth ,, ,, ,, ,, ,, . . .	29.5
Dark blue ,, ,, ,, ,, ,, . . .	32.1

L. Strachey[3] records that Florence Nightingale used to lay down the rule to Viceroys of India, on their departure to take up their job, that the windows of all hospitals in India must be kept open day and night, a practice which is wrong in the case of thick-walled buildings which are suitable for a hot country, as is shown by the following statement by H. E. Roaf:

'In solid buildings such as I was used to in Cairo the thick walls protected one from the heat, and one was accustomed to having the windows and shutters closed in the morning before it got hot (usually before 8 a.m.) and opened when it became cool again (somewhere between 5 p.m. and 6 p.m.). It was always a great relief to go into these cool, darkened rooms on a hot day. If my servant neglected to shut the rooms up it was most uncomfortable on a hot day. The air spaces were large and the thick walls did not get heated.

[1] On a warm sunny day the temperature inside a soldier's cloth hat was 98½°, a silk hat 86°, a Panama hat 80° F.

[2] P. Schmidt, *Arch. f. Hyg.*, **65**, 17 and 69.

[3] *Eminent Victorians.*

'Where we usually have a cool breeze and no thick walls it is better to open everything for the breeze and to keep the sun off by double roofs, verandahs, &c. Rush roofs protect from the sun yet allow free ventilation of air.'

Emission of radiant heat from clothing materials can be determined by means of a Leslie box full of water at boiling temperature, and a suitable thermopile and galvanometer. Taking the black side of the box as emitting 100,

a side covered with shiny silk emits	83·3
,, ,, ,, flannel ,,	108.7
,, ,, ,, cotton ,,	109.9

If the stuffs are made wet and the surface temperature kept the same, the emission is increased 39 per cent. A wet outer garment for this reason may lose more body heat in addition to evaporative loss.

If one puts the emission of radiant heat from the naked skin in a room at 15° C. at 100, it is reduced, says Rubner, by

a wool vest to 73 ;
,, ,, ,, and linen shirt to 60 ;
,, ,, ,, ,, ,, ,, and waistcoat to 46 ;
,, ,, ,, ,, ,, ,, ,, ,, and coat to 33.

The emission of the hairy parts is half that of the naked. Putting the emission of the naked skin at 100 in a room at 15° C.,

it becomes at 23° C.	69;
,, ,, ,, 29	56;
,, ,, ,, 32	31.

The absorption and reflection of sunlight by dark and light clothes is considered in Chap. VII. The following shows how a grey coat diffusively reflects less and absorbs more sunlight and reaches a higher surface temperature than the skin on a September calm sunny day. Thermopile arranged for indicating reflection of rays from surface.

Air 16·5, water 16·5, dry K.T. cooling power 12 :

		Deflection of galvanometer	Surface temperature
Cheek	shade	5	33° C.
,,	sun	15	34
Back of hand	shade	5·5	31·5
,,	sun	12·5	32
Grey coat	shade	4	22.7
,,	sun	9	36·5

The transference of water vapour from the skin through the clothes in a still atmosphere is due to diffusion through the clothes and to convection currents which escape at ventilation openings, such as the neck and end of the sleeves. In cold weather we shut up these openings and reduce convection currents by undergarments which overlap, by mufflers round the neck, gloves, &c. In summer the openings are made free and wide.

The open neck acts as a flue for the escape of body heat, and the close collars worn by men are disadvantageous compared to the free opening which women indulge in to display their charms.

Almond of Loretto School would never allow his boys to tightly confine the neck or feet.

Just as the flow of heat through unit area per unit time is numerically equal to the conductivity multiplied by the temperature gradient, so in the case of diffusion the transfer is directly proportional to the difference of concentration and the distance between the two elements of space containing the differing concentrations.

Horace Brown [1] says the phenomena of steady diffusion around a sphere are strictly analogous with those exhibited by a freely suspended and insulated spherical conductor which is electrically charged. The concentric shells of equal density of the diffusate correspond with the shells of equipotential in the dielectric surrounding the charged sphere, and the gradient of density measured along the lines of flux corresponds with the gradient of potential around the charged sphere measured along the lines of force. Moreover, the efficiency of the diffusive sphere is the exact analogue of the electrostatic capacity, both varying directly with the linear dimensions of the sphere.

'In a diffusive field the actual efficiency of a sphere functioning as an absorber or emitter of the diffusate varies directly with the radius of the sphere. Hence it follows that if a sphere is divided into any number of spheres n, of equal volume, and these are distributed in a similar field at such distances apart as not to interfere with each other, then the increased efficiency of the divided system will be represented by $n\sqrt[3]{\frac{1}{n}}$, when that of the original sphere is taken as unity. On the other hand, the increase in surface will only be represented by $\sqrt[3]{n}$, so that the efficiency increases as the square of the surface.'

Thus, if a sphere of unit volume is divided into 1,000 equal spheres, the surface extension over that of the original sphere is represented by 10, the diffusive efficiency of the whole subdivided system by 100.

The smaller the particles the more perfectly will diffusion maintain the critical concentration required, say, for some reaction by diffusion owing to the increasing convergence of the diffusive stream lines producing an ever-increasing gradient of concentration.

Thus minuteness of dimensions enables the living cell to supply its requirements from a medium of a much lower concentration of substrate than would be the case if the cell were of large dimensions; hence the importance of being little.

Just as the electric capacity of a plate is not considerably affected by cutting parts of it away and leaving an open framework, so also it is possible to block out a considerable portion of the cross-section of a diffusive column without materially altering the general static conditions on which the flow depends, or the diffusive efficiency.

A thin film of celluloid perforated with 100 circular apertures per sq. cm., each one of a radius of 0·19 mm., produced no sensible effect in obstructing the static diffusion of atmospheric carbon dioxide down a column of air, although the unperforate parts of the septum blocked out nearly 90 per cent. of the cross-section of the column.

[1] *Trans. Chem. Soc.*, 1918, **113**, 559.

The accelerated flow induced by each aperture neutralizes the obstruction produced by the unperforate parts. The stream lines become convergent and the density gradient increases in the immediate neighbourhood of the apertures.

So with the stomata of leaves and the stigmatic openings of the tracheae of insects, and clothes properly woven to give perforation suitable for evaporation.

Diffusive flow is very greatly accelerated through conical tubes, the flow being many times greater through a truncated cone than through a cylindrical tube of the same cross-section as the narrow end of the cone. This fact is probably of great importance in the alveolar regions of the lungs which lie beyond the region of the tidal air. The bronchiole expansions are conical, and thus accelerate diffusion from the alveoli.[1]

Hygroscopic water is attached to the surface of solids in the thinnest of layers. Dried protein, starch, wood, clothes, &c., imbibe about 12–15 per cent. of water from air of average humidity. The hygroscopic water depends on the relative humidity of the air, not on the temperature or absolute humidity. A greater or lesser velocity of air movement leaves the amount of hygroscopic water unchanged. The velocity of absorption depends on temperature and barometric pressure. In steam at 100° C. materials quickly become saturated.

Dry hair and clothing materials imbibe water with evolution of heat. The careful drying (airing) of clothes by the good housewife results, then, in greater warmth and comfort, for when the garments are put on they imbibe water vapour. So too with dry bedclothes. Putting the head under the sheets and breathing helps to warm up the bed by the bedclothes imbibing the exhaled water vapour.

On coming in out of dry, sunny, frosty air into a warm, humid atmosphere, water vapour both condenses on the cold surface of the clothes and is imbibed by the dry fibres, thus increasing the feelings of warmth. There is a schoolboy trick of drawing tight the sleeve and breathing through it, and so producing a hot sensation. On going from moist into cold and relatively dry air the evaporation of the 13–14. per cent. of water absorbed in the clothes increases the feeling of cold.

Horace Brown has investigated the imbibition of dry starch, malt, &c., and gave the following summary of his conclusions :

Nearly all organized substances, even when they contain no sensible pores, have the property of absorbing a relatively large amount of water, which penetrates between the molecules of the substance and causes it to increase in volume. The forces exerted by this penetration are very great, e.g. rock can be split by moistening dry wedges of wood driven into it, the method of quarrying used by the ancient Egyptians.

Heat of imbibition is observed when substances such as starch, cellulose, cellular tissue, &c., in a dry state, imbibe water, either on immersion or when submitted to air containing water vapour, which is at a higher partial pressure than the residual water in the substance itself. When equilibrium takes place, the evolution of heat ceases. The hydration of starch is accompanied by a condensation of volume,

[1] H. T. Brown, *Trans. Chem. Soc.*, 1918, **118**, 559.

and the evolution of heat runs *pari passu* with the condensation of volume.[1]

The evolution of heat is not due merely to a condensation of water on the surface of the starch, &c.; it is an expression of a real chemical action, which is reversible, since the starch, &c., can be brought back to the original state by drying.

A gramme of dry starch yields 20·08 calories on mixing with water.[2]

The contraction of volume may be compared with that observed when water and strong alcohol are mixed, or when a solid body goes into solution, and is more marked at the commencement of imbibition.

The imbibition of water vapour by clothing materials may be compared with the absorption of gases by charcoal. Hardwood charcoal heated red (in ways found to yield the best results) and allowed to cool will readily absorb gases even 600 times its volume.

Dewar found that when gases are condensed by charcoal the amount of heat liberated is considerably in excess of that which necessarily attends the passage from the gaseous into the liquid state,[3] thus:

	Molecular heat of liquefaction	Molecular heat of absorption
Hydrogen	238	1600
Oxygen	1664	3744
Nitrogen	1372	3684

When charcoal is brought into contact with a gas presumably its surfaces become coated with a layer of molecules, and this surface layer attracts other molecules, thus forming the equivalent of a liquid layer.

In the perfect gaseous state the molecules are free from mutual control. In the liquid state the molecules are in a state of what may be termed shifting association, their vibratory activity being so much reduced that they are able to cling together during an appreciable interval of time in virtue of the surplus or residual affinity with which they are endowed. A liquid, therefore, always has a certain attraction for the gaseous molecules of its own kind; the change from one state into the other may be regarded not as a mere physical but as a chemical change, inasmuch as it involves a change in the number of kinetic units, in other words, in molecular composition.

Masson and Richards wound 0·5–1·0 grm. of absorbent cotton round a thermometer bulb, dried it for 24 hours over phosphorous pentoxide, and then studied the increase of temperature indicated by the thermometer and the increase in weight of the cotton when suspended in air containing moisture.

Masson says, 'for practical purposes the heat liberated is directly proportional to the weight of moisture absorbed. This does not necessarily mean that there is nothing else to be considered than the simple heat of liquefaction. It may be so, but the condensation of vapour may be supplemented by some other process, either exothermic or endothermic.'

[1] H. Rodevald, *Ztschr. f. physikal. Chem.*, 1897, **24**, 2.

[2] Cf. *J. of Fed. Instit. Brewing*, 1899, **5**, 341; 1910, **16**, 120; *Trans. Guinness Res. Lab.*, 1908, **1**, 110–12.

[3] *Low-temperature Research at the Royal Institution.* Essay by H. E. Armstrong, Hodgkins Trust, 1909.

He suggests [1] that on immersion in water vapour condenses upon the cotton, the air adherent to the fibres preventing complete contact, and the heat gained is matched by an equal loss of heat by the adjacent water, the large heat capacity of the latter preventing any fall of temperature comparable with the observed rise.

With Richards [2] he concludes that cotton containing a definite proportion of moisture resembles an aqueous solution in that it exercises a vapour tension which is at different temperatures a constant fraction of that of pure water.

If w = weight of dry cotton employed.

p = actual pressure of water vapour in the atmosphere employed.

P = saturation pressure of water vapour at the same temperature.

M_A = weight of moisture absorbed by the sample of dry cotton of weight w after exposure in the apparatus till further absorption appears negligible.

M_E = the weight of moisture retained by the same sample after it has been supersaturated by exposure over water and then allowed to evaporate in the apparatus till further loss appears negligible.

M = arithmetic mean of M_A and M_E.

The value of M was found by Masson to depend upon the values of the fraction p/P.

With an atmosphere of given humidity M/w was a constant ratio.

Let t = time measured in minutes, from the moment of immersion of the cotton-covered thermometer.

θ = temperature of the same at the moment t, in degrees centigrade, its own initial temperature, which is the constant temperature of its environment, being taken as zero.

ϕ = maximum value obtained by θ in the course of the experiment.

r = the value of t at which $\theta = \phi$.

m = weight of water absorbed in time t.

It was found that the heat production was always proportional to the amount of hygroscopic absorption, therefore the above results may be given :

$$\left(\frac{d\theta}{dt}\right)_0 \text{ varies as } \left(\frac{dm}{dt}\right) \text{ varies as } p.$$

It was also found that in the case of the immersion of dry cotton in saturated vapour

$$\theta\left(t + \frac{r^2}{t}\right) = 2r\phi,$$

and it therefore follows that at any moment (t)

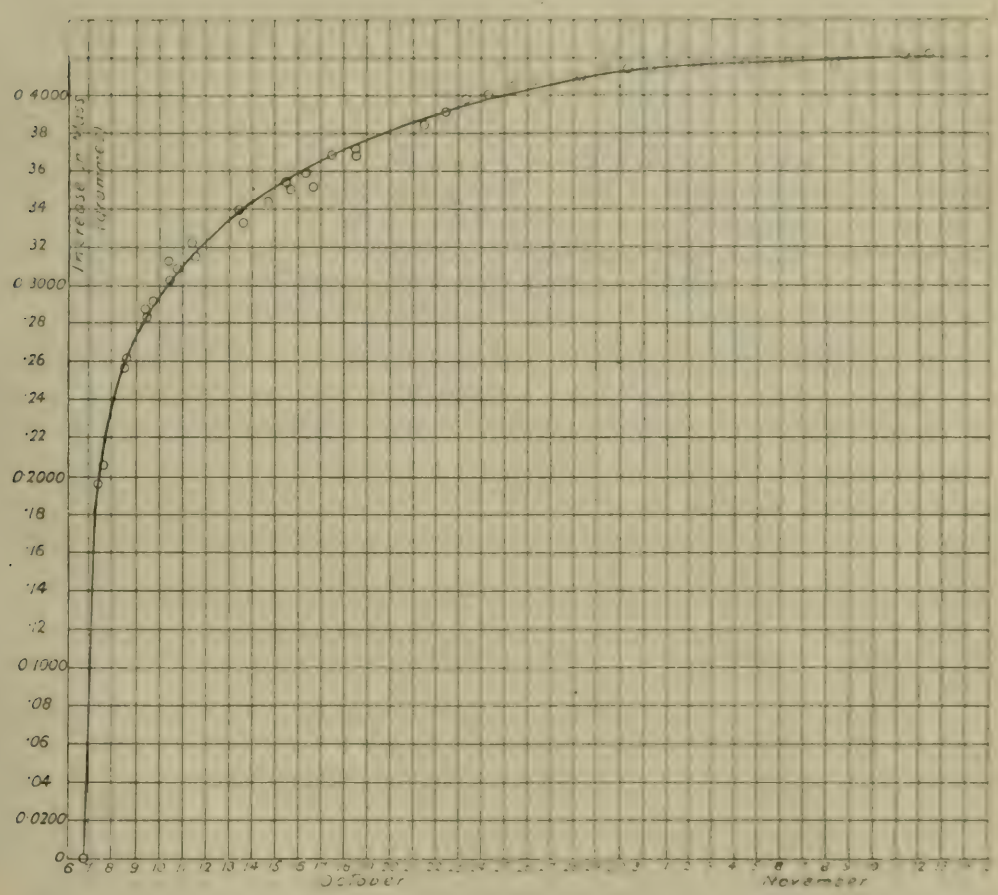
$$\frac{d\theta}{dt} = 2r\phi\frac{r^2 - t^2}{(r^2 + t^2)^2}.$$

Trouton [3] finds that the weight of water held by the material remains constant, however the temperature changes, so long as the relative humidity remains constant.

[1] *Proc. Roy. Soc.*, 1904, **A. 74**, 280.
[2] *Proc. Roy. Soc.*, 1906–7, **A. 78**, 413.
[3] *Proc. Roy Soc.*, 1905, **A. 77**, 392.

Rubner says 100 grm. of dry wool take up 25–28 grm. of water from saturated air, absorbing the water so that it is not sensible; 100 parts of dry silk take up 16·5; of dry cotton, 11·6. Cashmere increases 12 per cent. in volume on absorbing water.

Miss Hargood Ash and I studied the absorption of moisture of dry flannel suspended in a beaker over water. The piece of flannel was carefully dried in front of a gas fire until it gave a constant mass. The flannel was placed in a tube closed with a rubber cork for weighing. When a constant mass was obtained the roll of flannel was suspended by a silk thread from the beam of the balance, the thread passing through a hole in a cork fitted into a beaker, so that the roll hung


0·4000
38
36
34
32
0·3000
·28
·26
·24
·22
0·2000
·18
·16
·14
·12
0·1000
·08
·06
·04
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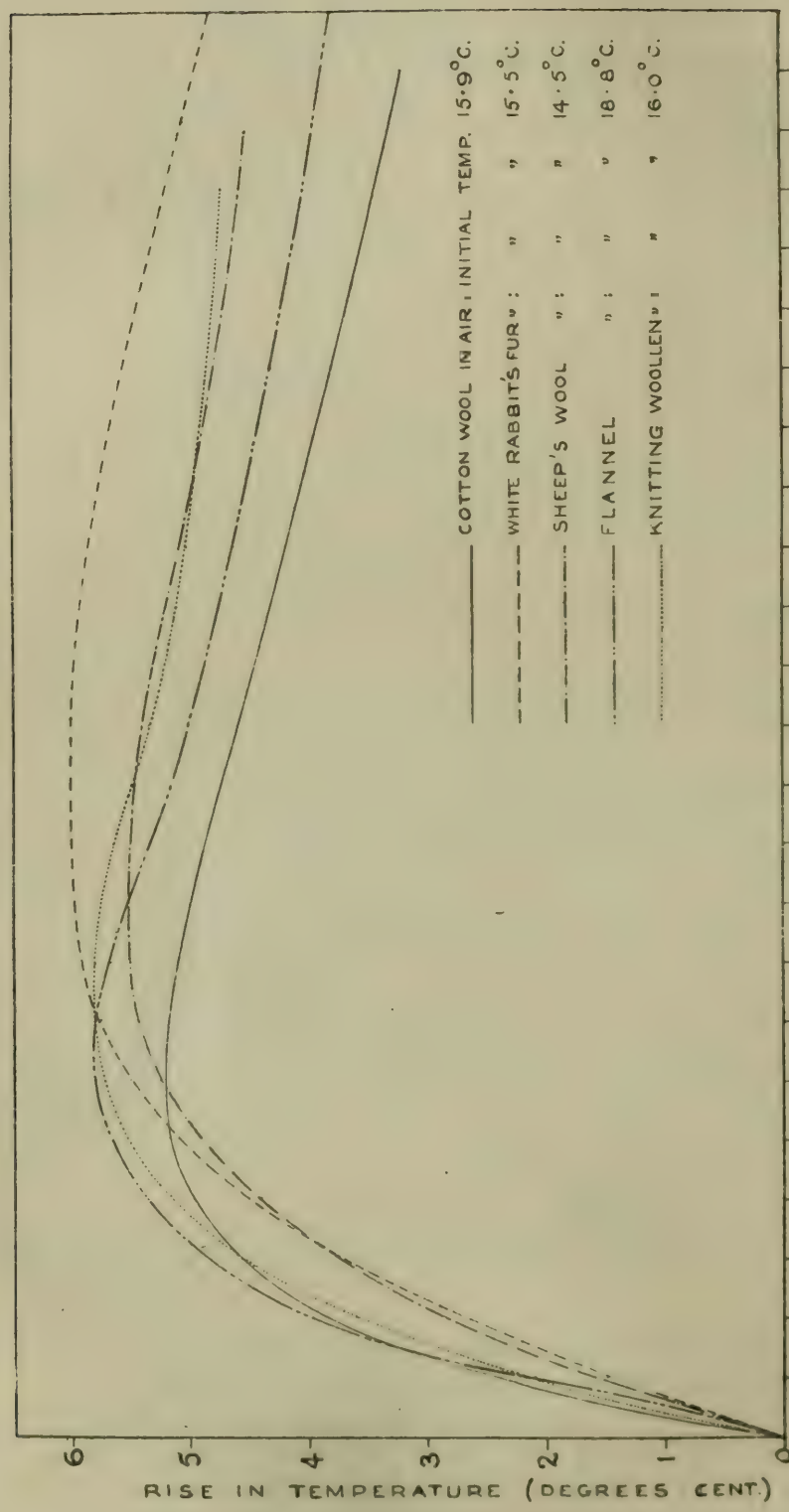


Fig. 34. Heat produced by dry clothing materials on absorption of water vapour.

We investigated the absorption of moisture by clothing materials, and the rise of temperature resulting therefrom, by a method suggested by Masson's experiments on this subject. Our results are given in Fig. 34. The material to be tested was dried in front of a gas fire, and then wound round the bulb of a mercury thermometer. It was tied round the stem above the bulb, and also gathered together and tied below the bulb. The thermometer had previously been passed through a rubber cork which fitted tightly into a short test-tube. The thermometer with the material attached was then kept in front of the fire for a few minutes to ensure dryness of the material, the thermometer reading about 50°–60° C. It was then introduced into the short test-tube (which had also been drying before the fire), the cork forming an air-tight stopper.

The tube containing the thermometer and material was then suspended in the room and left until the thermometer reading was the same as that of a control thermometer suspended near to it. This cooling process was often hastened by partly immersing the tube in cold water until the readings of the two thermometers were nearly equal. When the enclosed thermometer indicated that the material had attained the room temperature the tube was slipped off the cork and half-minute readings of the temperature taken.

In the graphs (Fig. 34) the rise of temperature is given in centigrade degrees, the initial temperature being that of the surrounding air.

The material was wrapped about three times around the bulb. In the case of the knitting wool this was wound on. The rabbit fur was with the hair, not the skin part, next the thermometer.

A thermometer treated in exactly the same way, but with nothing round the bulb, showed no rise of temperature when taken out of the tube.

The thermometer was in each case suspended in an ordinary room, no special precautions with regard to sheltering being taken.

Since the drying was not necessarily the same in all cases, the results obtained are not absolutely comparable.

With respect to the mass of moisture absorbed from the atmosphere, it was found that 1·066 grm. of dry flannel had increased in weight by 0·0906 grm., and was still gaining weight when the experiment was stopped.

With a dry three-layer thick garment on, in spite of the cooling rate, the thermometer rises in 5 minutes 5° and 6° C., say 10° F., and the increase of temperature is maintained for many minutes, showing the very comforting amount of warmth well-aired dry clothes must afford the body when put on.

That the heating of the clothing material is not merely due to the condensation of water vapour on the fibres and the setting free of the latent heat of evaporation, is shown by the fact that on immersing the material in water both the material and the water become heated. This heat we have measured calorimetrically.

The experiments were carried out as follows:

The material was dried, either in front of a gas fire or in a desiccator with sulphuric acid, together with a short test-tube and rubber cork. The material was then introduced into the tube, after being rolled up and tied with a piece of silk. A thermo-junction made of thin copper

and eureka wire was quickly pushed into the middle of the roll and the cork tightly inserted in the tube. The tube was suspended in a copper calorimeter containing distilled water, the copper calorimeter was suspended in a large outer vessel containing air by fine silk thread. In the water was a similar thermo-junction to that in the material. The cold junctions of both thermo-couples were kept in melting ice. A thermometer inserted in the outer vessel gave the air temperature.

Before beginning an experiment it was ascertained that both couples gave the same deflections. The tube containing the dried material was left in the water until the two couples gave the same readings, when it was quickly taken out, the roll of material with the junction in it extracted and placed in the water. Time readings were taken of both thermo-couples; owing to the fact that only one galvanometer was available, these are not simultaneous. The galvanometric scale was calibrated in the usual way.

Fig. 35 shows the results obtained for cotton material.

The slight rise given by the clothing material junction, before

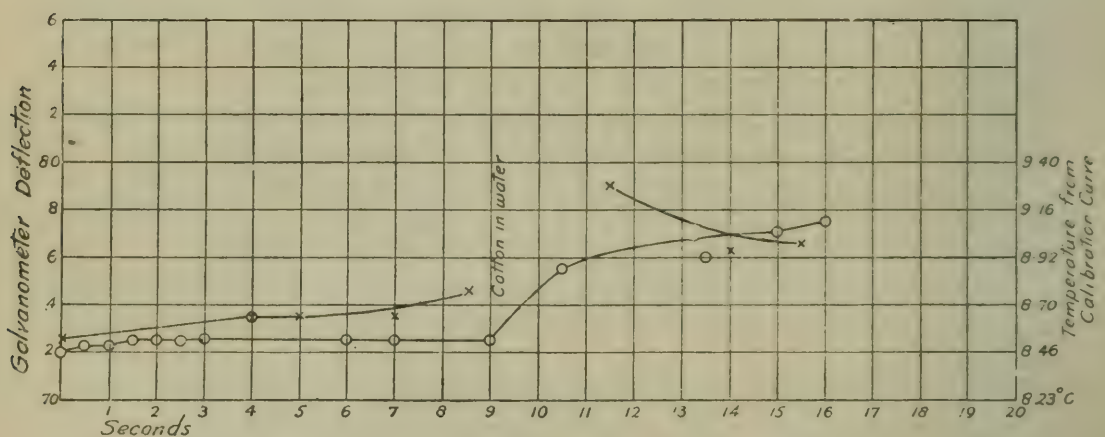


Fig. 35. Calorimetric investigation of the heating of dry cotton on immersion in water. Upper curve temperature of cotton, lower curve temperature of water.

introduction into the water, was probably due to a trace of moisture entering the tube along the cotton-covered wires which from the thermo-couple passed between the tube and the cork.

The cotton temperature apparently fell at the end of the observation below the water temperature; this was due to one of the cold junctions getting out of the ice, both registering alike when this was remedied.

The figures obtained were:

Mass of copper calorimeter = 39·64 grm.
Mass of cotton = 1·84 „
Mass of water = 65·18 „

The increase of temperature of the water was 9·1–8·5 = 0·6° C.

Therefore, neglecting the heating of the cotton and taking the specific heat of copper as 0·09, we have

Heat produced = (39·64 × 0·09 + 65·18) 0·6.
„ „ = 41·3 calories or 22·4 calories per grm.

Horace Brown found 20·08 calories for starch.

The air temperature rose from 9·2°–10·2° during the experiment.

In the case of the flannel the figures were not taken; but the calorimeter used was the same and the mass of water very approximately so; in this case the rise of temperature of the water was (11·5°–11·2°) = 0·3°.

The air temperature rose from 11·5°–12·4° during experiment.

Soaked in water materials take up more water the more air they hold when dry. 100 grammes take up according to Rouget de Lisle:

Flannel	Cotton wool	Silk	Linen	
200	100	95	75	Grammes of water when wrung out by hand.
100	60	50	40	,, ,, ,, ,, machine.
60	35	30	25	Centrifuged.

Rubner found 1,000 grammes of

Knitted silk	take up	1,409	grammes	of water
,, cotton	,,	1,180	,,	,,
,, wool	,,	1,400	,,	,,

H. E. Roaf found the following percentage absorption of materials wrung out by hand: Mosquito net, 133; Japanese crêpe, 140; China silk, 101; cotton, 132; flannel, 141; drill, 93; light serge, 102.

To investigate the water vapour penetrating power of clothing materials the following experiments were carried out:

Into a beaker of 520 c.c. capacity there was put a dish containing 10 c.c. of concentrated sulphuric acid. The dish and acid were weighed. The top of the beaker was covered with a sheet of material which was fixed in place by a rubber band. The beaker was placed in a chamber and left 21 hours, when the dish and acid were again weighed.

The amount of water taken up was:

Cellular cotton	3·601 grammes
Flannel	3·160 ,,
Cotton shirting (1)	3·234 ,,
,, (2)	3·467 ,,
,, (3)	3·731 ,,

Water was then put in the dish and weighed, and acid put in the chamber to keep the air therein dry.

The amount of water lost from the dish in 21 hours with the various covers was:

Cotton cellular	5·648 grammes
Flannel	5·893 ,,
Cotton shirting (1)	6·385 ,,
,, (2)	5·799 ,,
,, (3)	5·561 ,,

The covers also lost weight thus:

	Weight of cover	Loss in weight
Cellular cotton . . .	5·084	0·160
Flannel	7·634	0·372
Cotton shirting (1) . . .	10·257	0·353

It may be concluded that all these materials allow water vapour to penetrate with about equal ease.

Wetting has the following effect on the porosity of materials:

1,000 parts	Pore volume, dry	Pore volume, wet
Flannel	923	803
Cotton flannelette	888	723
Knitted silk	832	501
„ wool	833	612
„ cotton	747	617
„ linen	833	318
Smooth cotton	520	0

Thus a woven highly porous material preserves its air-holding properties very largely when wet.

Rubner cites the following: The clothing of a German soldier weighs dry 4,850 grm., wet 8,750 grm., taking up then 3,900 grm. of water. To evaporate this amount of water 2,300 calories are necessary, the day's heat-production of a resting man. Hence the enormous heat loss due to wet clothes, a loss which is of no consequence to a man heated by exercise, but of great consequence to one sitting still in a wind.

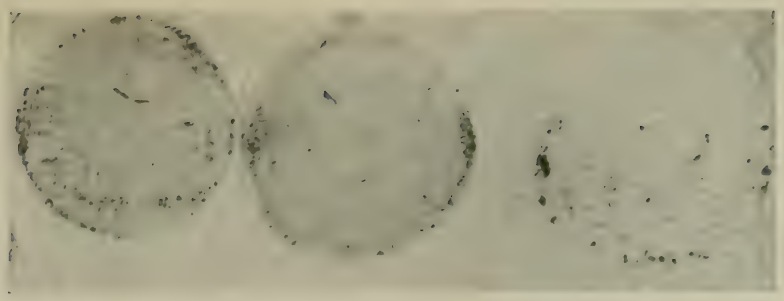
Wet materials stick to the skin, flannel and knitted material least, smooth materials most, as is shown in Figs. 36 and 37, which demonstrate the result of some experiments carried out with Flack. The less the adherence, the greater the volume of entangled air and the heat-retaining power of a wet material, e.g. flannel.

The heat-conductivity is increased by wetting in the following proportion (Rubner):

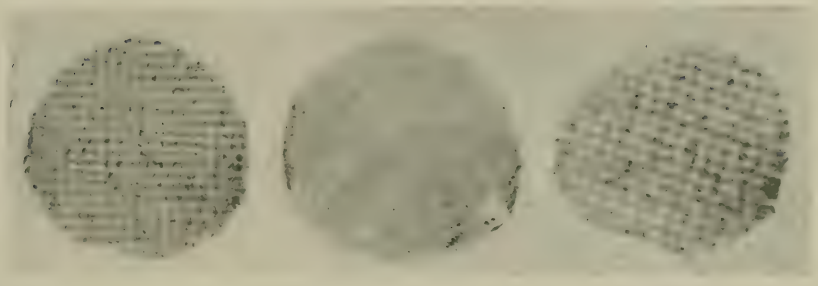
Flannel	Dry = 1	Wet	1·56
Knitted wool	„		2·19
Smooth cotton	„		3·39

Far more, then, in the case of smooth cotton materials.

Cool, damp atmospheres are chilly owing to the dampness of the clothes and increased loss of heat by conduction and evaporation. Water birds oil their feathers to keep them dry. The arrangement of fur of animals is such as to shoot off the rain and keep the inner part and skin dry. Ducklings and chicken wet through easily die of cold. The importance of securing dryness may be illustrated by the behaviour of cormorants evolved by the hard necessity of succeeding in the struggle for existence. 'All along our coast there is at various intervals a rock popularly distinguished in the neighbourhood by the name of "Shag Rock". Such a rock is generally low, isolated, and situated at a safe distance from land, or, if near the shore, is close to the base of a steep cliff. Hither the cormorants, when their hunger is appeased, repair for the threefold purpose of resting, digesting their food, and drying their wings. The process of digestion is soon completed, but the time consumed in drying their thoroughly drenched wings depends on the amount of sunshine and air-movement. Of these, whatever they may be, they know how to avail themselves to perfection. They station themselves on the highest ridge of the rock, wide apart, and in a row, so as not to screen one another, raise their bodies to their full height, and spread their wings to their utmost extent. No

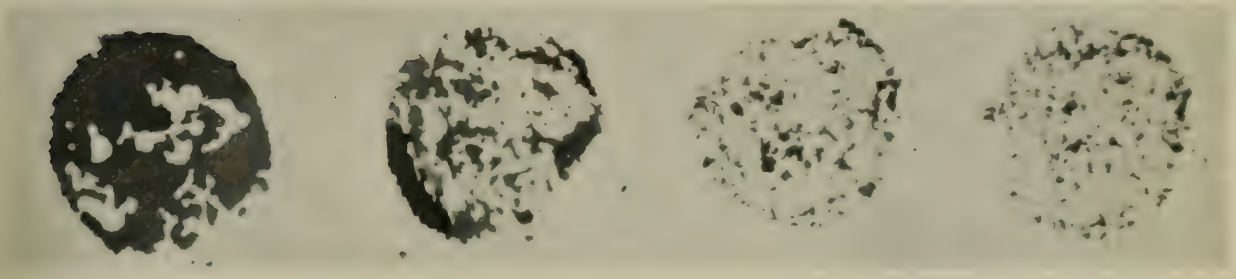


Flannelette. Angola Cotton. Flannel.

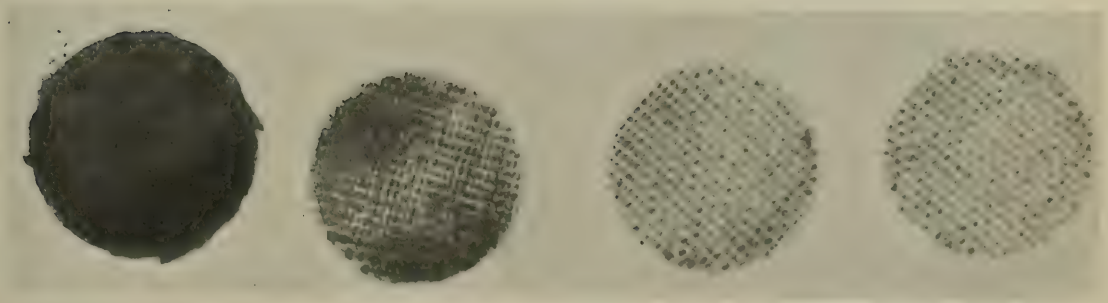


Cotton Shirting. Silk. Cotton, open mesh.

FIG. 36. Disks of materials were moistened with a dye (the excess squeezed out). Laid on paper, a photographer's roller was lightly passed over them.



Flannel.



Cotton.

FIG. 37. A disk of each material moistened with dye and laid on paper, a roller was then lightly passed over each and the 1st, 10th, 20th, and 30th impressions recorded.

laundress is more cunning in the exercise of her vocation. Indeed, they can hardly fail to recall the idea of so many pairs of black trousers hung out to be aired!'[1]

Begonie determined what he called the coefficient of utility of garments, clothing a copper vessel filled with water at 37° C. and measuring the time it took to cool 10° C. in an atmosphere at 12° C. He found the following:

Flannel	1·75
Pyrenese wool	2·5
Fur pelisse.	4·5

The heat-retaining efficiency can be measured by the kata-thermometer.

In still air clothes make a much smaller difference to cooling power than in wind. In the still-air chamber, temperature 13·4° C., the dry kata gave the following:

	Cooling power	Lessening of cooling power by clothes
Naked	6·5	
With thick knitted woollen glove . .	4·9	24½ per cent.
Ditto and thin rubber cover . . .	4·0	38
On the roof exposed to a light breeze, temperature 5·2° C.		
Naked	22	
With thick woollen glove . . .	10	54½ per cent.
Ditto and thin rubber cover . . .	7	68

On passing, then, from a still room at 13° C. to a light breeze at 5° C. out of doors, the heat-protecting power of the woollen glove is more than doubled.

Table XV shows the difference observed between the naked dry kata and the kata clothed in the finger-stall of a thick, white, knitted wool glove. When the clothed kata readings were about or above 20 in very cold winter weather, two overcoats, thick gloves, and a muffler were worn by the observer, who walked about to keep warm and often took shelter from the wind behind a wall while observing. When the clothed kata readings were about 10 or a little above it, on sunny very bracing days, a rug and an overcoat were required for sitting out. On pleasantly warm days ideal for sitting out on the cliff, the clothed kata gave readings of about 3 to 5, the readings being taken in the sun. The clothed dry kata roughly gives about half the cooling power of the naked dry kata in the conditions recorded in the table.

In Table XVI are seen the readings taken with naked and clothed dry kata in Sunderland in March and July. The clothed kata had a double finger-stall, (1) of cellular cotton shirting, (2) of flannel. Here again out of doors exposed to the wind the dry kata cooling power is reduced to a half or more by the clothes. The ordinary room readings of the naked kata are given for comparison, so that it may be seen how the clothes approximate the rate of cooling out of

[1] C. A. Johns, *British Birds in their Haunts*, 1909, 166.

doors of the clothed kata to room readings of the naked kata. This is not the case on many days, especially in March, when an extra garment beyond the two, or one more wind-proof, is required. Such readings indicate how clothes bring about still-air conditions—it is obvious that indoors the clothed body may be surrounded by too still an atmosphere, one which becomes warmed and made humid by the skin, giving also a monotony of stimulation alike to naked and clothed parts. Out of doors the wind, by ventilating the clothes, prevents a too stagnant atmosphere, and there is a wide difference in the stimulation of the naked and clothed parts.

Table XVII shows clothed and naked kata readings in a sheltered Guildford garden. The rate of cooling of the naked and clothed parts is not so widely different in the less windy atmosphere.

In taking readings of the clothed kata it must be borne in mind that hygroscopic property of the clothing materials is great, and the relative dampness of the clothes may affect the readings. It is advisable to warm the kata and let it cool with the glove on it two or three times before taking readings.

TABLE XV.

Cooling power		
Dry kata	Dry kata, clothed	
56.3	24	On cliff or shore, sea coast; very cold days; two coats and overcoat worn, muffler and gloves.
53.2	24	
43.5	20.4	
47	22.8	
43.5	22.1	
56.3	25.2	
40	18	
41.6	20	
40	20	
53.2	22.8	
16.6	9.6	Sunny; very bracing; rug and overcoat for sitting.
22	10.2	
25	10.5	
23.5	10.9	
21.5	11.2	
23	14	
27	11	
27	13.5	
16.6	8	
22.7	10.6	
20.6	11.8	
9	4.9	Sunny; pleasantly warm days; ideal for sitting out on cliff.
6.4	3.5	
7.9 (12.8 shade)	4.2 (8.4 shade)	
4.9 (9.4 ,,)	2.8	
8 (12.3 ,,)	4.1	
4.7 (7.8 ,,)	2.6 (3.7 ,,)	
5	2.8	
9.6	5.3	

TABLE XVI.—*Comparison of Coated and Naked Dry Kata. Sunderland.*

March			July		
Outdoors naked	Outdoors coated	Ordinary room naked	Outdoors naked	Outdoors coated	Ordinary room naked
28·79	11·76	7·68	13·25	7·59	7·26
22·98	11·81	7·45	12·47	7·08	6·31
17·28	9·52	8·79	21·98	7·57	6·96
18·55	10·27	8·05	13·69	7·93	7·41
33·4	14·23	8·61	22·77	10·52	6·36
33·85	15·18	9·52	23·63	11·23	7·90
31·32	12·4	8·95	18·15	7·78	6·68
25·30	10·93	8·41	19·27	8·46	6·79
30·55	12·27	6·07	19·42	8·43	10·27
24·32	10·98	7·73	24·32	9·86	8·13
33·4	20·04	11·03			
24·56	9·21	7·64			

TABLE XVII.

Sheltered Guildford garden.

June		August		October	
Outdoor Dry kata		Dry kata		Dry kata	
naked	clothed	naked	clothed	naked	clothed
10·8	6	6·2	4·5	12·8	5·2
9·9	6·5	6·7	4·1	10·3	6·0
16·3	7·5	9·8	5·0	20·4	7·5
14·1	6·7	9·6	5·2	8·6	7·3
15·1	6·2	8·0	5·5	13·6	7·4
13·2	6·4	9·4	5·4	8·5	5·0
10·6	6·2	7·0	4·9	11·4	6·5
10·6	7·4	8·8	4·3	11·4	7·2
10·3	6·4	7·6	4·1	15·1	6·8
11·1	7·2	9·0	5·6	12·8	8·1
18·4	8·5	6·3	4·2	10·3	7·7
14·1	6·6	9·2	4·0	14·6	8·0
		6·4	4·7	17·6	7·8

The protective effect of different garments is shown by the following figures recorded by M. Flack and L. Hill:

TABLE XVIII.—*Dry Kata Cooling Power in Room.*

Knitted wool	Silk	Open mesh cotton	Close mesh cotton	Suède	Naked	Temperature
4·8	6·0	6·1	5·7	5·2	6·1	15·5–17° C.
In wind: 1 m. per sec. approx.:						
7	11·1	12	11·1	10·8	15	18–18·5
In wind: 6 m. per sec. approx.:						
10	17·5	18	17	15	24	17–18

With the finger-stalls fitting directly on to the kata the wool glove entangling the most air in its mesh is the best, and the protection it affords is much greater in wind (10 clothed, 24 naked) than in still air (4·8 clothed, 6·1 naked).

A close-fitting, light, wind-proof cover next the skin does not protect because the material is a good conductor. A 'doped' silk or thin rubber

cover stretched lightly over the dry kata scarcely delays its cooling at all. Put on loosely so as to confine a layer of air and the protective effect becomes great. The nature of the fibre of the material makes no difference. The heat-retaining power depends wholly on the immobilization of air. With loose-fitting gloves entangling air and in a wind of 3·5 mm. per sec. approx. (wind tunnel) the dry kata's cooling power was as follows:

Naked	16·5
Clothed, thick knitted woollen glove	7·1
Woven cotton, close mesh	9·5
Chamois leather	9·0

In a wind of 12 m. per sec. approx.:

Naked	26·6
Clothed, thick knitted woollen glove	11·9
Woven cotton, close mesh	12·9
Chamois leather	10·4

Table XIX shows the effect of two garments on the cooling power of the dry kata when fully exposed to the wind in November and December, the garments being an inner cotton and outer woollen, and of these two garments together with a wind-proof material. The two garments halve the dry kata's rate of cooling. The three garments generally bring its rate of cooling down to one-third, to rates which pertain to the naked kata indoors in this country, or out of doors in tropical humid climates.

TABLE XIX.

Date 1917	Surface temp. of cheeks		Cooling power of kata (dry)			Temperature		East Coast fully exposed to wind
			Naked	Clothed	Clothed wind-proof	Dry	Wet	
Nov. 9	23° C.	21·5° C.	23·7	13·2	7·5	9·4	7·2	Fine, strong wind.
10	—	19	19·8	12·5	7·3	6·7	6·1	Cloudy, N.
13	—	19	20·6	12·2	8·4	7·2	6·9	Thick mist, cold breeze
15	21·5	20	21·6	14	7·3	6·1	5·6	Sunny, still.
18	20	19·5	18·2	11·4	6·7	11·4	8·9	Cloudless, breezy.
20	25·5	25	19·8	9·5	5·3	12·5	10·3	Fine, breezy, NW.
21	—	24	16·4	8·6	5·7	12·8	11·9	Cloudy, NW.
23	22·5	21·3	19·8	11·7	6·6	11·4	9·4	Sunny, NW.
24	—	18·3	33·9	17·2	9·5	12·2	10·6	Fine, heavy wind W.
26	—	17·5	21·6	10·9	8	2·8	1·7	Misty sun, cold.
28	26	25	19·8	8·7	5·3	13·6	11·7	Bright sunshine, wind W.
Dec. 2	16·5	16	28	14·3	8·3	3·3	0·6	Cloudless, cold, NW.
4	21	20·5	19	11·7	7·3	2·8	1·7	Bright sun, still misty.
6	20	19·5	21·6	10·3	7·1	4·4	3·3	Cloudless, misty.
7	—	20	26·4	12·9	9·3	10·6	9·4	Cloudy, misty.
11	27·2	25·5	14·4	9·5	6·4	6·4	4·7	Sunny, calm.
13	—	23·5	15·3	9·7	5·7	8·6	8·3	Cloudy, light wind.
15	18·2	17·5	26·4	14·3	8·1	5·0	3·3	Cloudless, moderate wind.
17	—	18	33·9	11·7	7·8	2·8	0·3	Fine clouds.
20	22	21·5	14·8	9·7	7	0·6	0·6	Cloudless, calm, misty.
22	22	21	12·2	14·7	8·7	3·9	1·7	Calm, sunny.
24	23·5	23	15·8	9·1	7·1	5·0	5·0	Sunny, damp.
28	—	21	17·6	12·5	7·8	3·3	2·2	Sunny, snow on ground.
30	—	20	33·9	16·6	9·3	0·8	0·3	Cloudy, NE., cold.
Jan. 6	—	18	25	15·1	9·2	1·7	0·6	Cloudy, SW., cold.

Table XX shows dry kata readings taken by H. E. Roaf with close-fitting garments.

TABLE XX.—*Close-fitting Bag, Dry Kata* (observer, H. E. Roaf).

Material	Slowest rate of fan			
	16. 1. 17		3. 2. 17	
		per cent.		per cent.
Uncovered	13·5	100	14·7	100
Mosquito net	12·2	90·0	10·4	70·8
Japanese crêpe	—	—	10·0	68·1
China silk	11·3	84·5	10·6	72·2
Thin flannel	9·6	71·1	10·6	72·2
Drill	—	—	—	—
Serge	—	—	—	—
Composition	—	—	9·6	65·7
Uncovered still air	5·68	42·2	5·0	34·0
Dry bulb	61° F. (16·1° C.)		60·5° F. (15·8° C.)	

Material	Medium rate of fan					
	28. 1. 17		17. 2. 17		3. 3. 17	
		per cent.		per cent.		per cent.
Uncovered	22·7	100	22·7	100	24·9	100
Mosquito net	19·2	84·8	17·2	76	17·3	69·0
Japanese crêpe	17·1	76·0	16·1	71	15·1	60·8
China silk	18·5	81·5	17·8	78·5	16·6	66·7
Thin flannel	15·6	68·8	15·6	69·0	14·7	59·0
Drill	—	—	—	—	15·6	62·5
Serge	—	—	—	—	12·5	50·0
Composition	—	—	15·6	69·0	13·5	54·1
Uncovered still air	5·37	23·7	5·91	26·2	5·67	22·8
Dry bulb	62° F. (16·7° C.)		59° (15°)		61° (16·1°)	

Material	Fastest rate of fan							
	21. 1. 17		11. 2. 17		25. 2. 17		17. 3. 17	
		per cent.		per cent.		per cent.		per cent.
Uncovered	27·8	100	22·7	100	26·3	100	17·8	100
Mosquito net	18·5	64·4	17·8	78·7	19·2	73·2	12·8	71·8
Japanese crêpe	17·8	64·3	14·3	62·8	16·1	61·5	13·5	61·0
China silk	20·0	72·0	16·6	73·3	19·2	73·2	10·9	75·5
Thin flannel	17·8	64·3	15·1	66·7	14·7	56·0	10·0	56·0
Drill	—	—	—	—	16·7	63·3	12·4	68·2
Serge	—	—	—	—	13·2	50·0	9·8	55·0
Composition	—	—	15·1	66·7	13·5	51·5	10·0	56·2
Uncovered still air	5·93	21·3	5·31	23·4	5·38	20·5	3·72	20·8
Dry bulb	58° F. (14·4° C.)		62° (16·7°)		62° (16·7°)		73° (22·8°)	

The slowest rate of fan gave a wind velocity of about 1 m. per sec.
The medium " " " " 6 "
The fastest " " " " 8 "

Observations made by M. Flack and L. Hill out of doors on the East Coast cliff showed the cooling power exerted by the wind on the wet kata is reduced by enclosure in a dry garment, this being separated by a wire frame from contact with the wet kata, as follows:

Garment (dry)	Lessening of cooling power (wet kata)
Flannel, Angola flannel, Flannelette, Close-meshed thick cotton shirting	67 per cent.
Close-meshed cellular cotton shirting	63 ,,
Pure silk	60 ,,
Knitted sweater	57 ,,
Open-meshed cellular cotton shirting	50 ,,

The following shows the results of protecting the wet kata in still air, with a coat (1) dry, (2) wet, on a wire frame enclosing the kata, and (3) wet, touching the kata:

Material of clothing	Range of temp. of air	No coat		Dry coat on frame		Wet coat on frame		Wet coat on kata	
			per cent.		per cent.		per cent.		per cent.
Knitted wool	15–16° C.	14·4	100	9·3	64	11·2	78	11·7	81
Cotton twill	14–14.5° C.	15·3	100	9·7	63	10·9	71	14	91
Muslin	11·5–12·8° C.	16·2	100	10·7	66	12	74	14·8	91

In wind a close-meshed *wet* garment separated by a wire frame from the kata is almost as protective as a dry one in preventing the escape of the warm moist air from the surface of the wet kata. An open-meshed garment is less so. Thus:

Garment (wet) on wire frame	Lessening of cooling power (wet kata)
Close-meshed thick cotton shirting	63 per cent.
Flannel	57 ,,
Sweater	40 ,,
Open-mesh cellular cotton shirting	30 ,,

Thus an overcoat wet with rain will obstruct the loss of body heat from the body when marching almost as much as a dry one; experience of one's feelings confirms this. A raincoat buttoned up to keep out the wet is very hot to walk in, and lessens one's pace on a windy, rainy day; far more so on a wet, calm day.

The power of a single garment to lessen the rate of cooling of the wet kata exposed to wind from a fan is shown in the following tables of observations taken by H. E. Roaf. The kata was surrounded by a loose bag 6 cm. in diameter stretched over a wire frame, or by a small bag separated from the wet kata by a minimum air space, i.e. a spiral of galvanized wire which just prevented contact. The small bag allowed better ventilation. The only material which allows a good draught through is mosquito netting, and even this may lower the cooling power 10–20 per cent. Flannel or drill cut down the cooling power 40–50 per cent., and even 70–75 per cent. in the case of the fastest rate of fan and the large bag. The diminution of cooling power is much less if the garment is wet and in contact with the wet kata (comparable with sweat passing right through a garment from the skin and wetting the clinging garment). The cooling power exerted on the wet kata is lessened not more than 5 per cent. by a clinging wet garment of mosquito netting or china silk, about 10–20 per cent.

by clinging wet Japanese crêpe, and 30–40 per cent. by clinging wet thicker materials which are loose in texture and contain entangled air. Open-mesh stuffs allow evaporation, while the close-meshed entangle vapour. On the other hand, close-mesh stuffs prevent penetration of sun rays, while open-mesh allow penetration. There has, then, to be a compromise between the two. An engineer told me he got most comfort in India by wearing a wide-meshed net material under his cotton shirt; this by holding off the shirt permitted ventilating currents of air to carry away vapour.

TABLE XXI.—*Large Bag 6 cm. in diam. over Wet Kata-thermometer* (H. E. Roaf).

Material	Slowest rate of fan				Medium rate of fan					
	16. 1. 17	per cent.	3. 2. 17	per cent.	29. 8. 16	per cent.	28. 1. 17	per cent.	1. 2. 17	per cent.
Uncovered	29·3	100	31·2	100	33·3	100	49·9	100	45·3	100
Mosquito net	22·7	77·3	29·4	94·2	29·4	88·2	35·6	71·6	38·5	84·5
Japanese crêpe	13·1	44·7	13·9	45·5	16·7	50·0	29·4	58·8	25·0	55·0
China silk	12·8	43·6	13·9	45·5	10·4	31·3	20·0	40·0	17·2	38·0
Thin flannel	11·3	38·6	13·9	45·5	10·2	30·7	17·8	35·7	16·1	35·5
Drill	11·9	40·5	13·1	42·2	9·62	28·7	13·9	27·7	13·9	31·6
Serge	11·1	38·0	11·1	35·7	9·62	28·7	14·3	28·7	13·1	29·0
Still air	13·5	46·2	15·13	48·5	8·76	26·3	14·3	28·7	14·7	32·3

Material	Fastest rate of fan			
	21. 1. 17	per cent.	1. 2. 17	per cent.
Uncovered	45·5	100	49·9	100
Mosquito net	41·7	91·7	41·7	83·5
Japanese crêpe	29·4	64·7	22·6	45·5
China silk	23·8	52·4	16·6	33·4
Thin flannel	16·6	36·7	16·6	33·4
Drill	15·1	33·0	12·5	25·0
Serge	13·5	30·0	12·5	25·0
Still air	15·1	33·3	14·2	28·5

TABLE XXII.—*Small Bag separated from Wet Kata-thermometer by Minimum Air Space* (H. E. Roaf).

Material	Slowest rate of fan		Medium rate of fan		Fastest rate of fan			
	28. 2. 17	per cent.	3. 3. 17	per cent.	25. 2. 17	per cent.	17. 3. 17	per cent.
Uncovered	33·4	100	55·5	100	49·9	100	45·5	100
Mosquito net	29·5	88·2	45·5	81·8	41·7	83·5	41·7	91·5
Japanese crêpe	20·8	62·5	31·2	56·2	35·6	71·7	33·3	73·5
China silk	20·0	60·0	26·3	47·3	31·2	62·5	27·7	61·0
Thin flannel	17·8	53·5	23·8	42·8	29·5	58·8	27·7	61·0
Drill	16·7	50·0	21·7	39·3	29·5	58·8	20·8	45·8
Serge	15·2	45·5	18·5	33·3	22·7	45·7	19·2	42·3
Composition	14·3	43·0	19·2	34·6	27·7	55·7	18·5	40·3
Still air	14·3	43·0	15·1	27·3	14·7	29·5	13·1	29·0

TABLE XXIII.—*Close-fitting Bag covering Wet Kata* (H. E. Roaf).

Slowest rate of fan

Material	16. 1. 17		3. 2. 17		28. 2. 17	
		per cent.		per cent.		per cent.
Mosquito net	29·4	100	31·2	100	33·4	100
Japanese crêpe	—	—	27·8	89·0	29·5	88·2
China silk	31·2	106	29·3	94·2	31·3	94·0
Thin flannel	22·6	77·3	23·8	76·3	27·7	83·5
Drill	—	—	—	—	25·0	75·0
Serge	—	—	—	—	19·2	57·5
Composition	—	—	20·8	66·8	21·7	63·5
Still air	13·5	45·8	15·1	48·4	14·3	42·8

Medium rate of fan

Material	28. 1. 17		17. 2. 17		3. 3. 17	
		per cent.		per cent.		per cent.
Mosquito net	49·9	100	45·5	100	55·5	100
Japanese crêpe	41·7	83·5	41·6	91·7	41·7	78
China silk	49·9	100	45·5	100	45·5	81·8
Thin flannel	38·5	77	35·7	78·6	35·7	64·2
Drill	—	—	—	—	38·5	69·3
Serge	—	—	—	—	29·4	53·0
Composition	—	—	27·7	61·2	31·2	56·2
Still air	14·3	28·7	14·7	31·0	15·1	27·3

Fastest rate of fan

Material	21. 1. 17		11. 2. 17		25. 2. 17		17. 3. 17	
		per cent.		per cent.		per cent.		per cent.
Mosquito net	45·4	100	49·9	100	49·9	100	45·5	100
Japanese crêpe	38·5	85	38·4	77	41·7	83·5	35·6	78·5
China silk	45·4	100	45·4	91	49·9	100	45·5	100
Thin flannel	35·7	80	35·6	71·4	38·5	77·3	41·7	91·5
Drill	—	—	—	—	38·5	77·3	35·6	78·5
Serge	—	—	—	—	29·5	58·5	29·3	64·8
Composition	—	—	29·3	59·0	31·2	62·5	27·7	61·0
Still air	15·1	33·3	14·22	28·5	14·7	29·5	13·1	29·0

M. Flack and I made a large number of observations on kata cooling powers taken under the shirt while sitting dressed in shirt and pants out of doors on the East Coast cliff in the summer. We found it made little difference whether the shirt was cellular cotton or flannelette or flannel, the protection from cooling depending on the restriction of ventilation, not on the nature of the material. This conclusion is confirmed by the following observations taken in Egypt by H. E. Roaf. The effect of double garments in lessening the wet kata cooling power is made evident.

TABLE XXIV.—*Observations by H. E. Roaf (Egypt).*

Wet kata cooling power under clothes.	Per cent. of exposed wet kata cooling power	Clothes
7·45	39	Thin shirt and thin vest
7·7	34	,, ,,
8·0	24	,, ,,
6·0	37	,, ,,
10·0	29	,, ,,
14·0	44	Thin flannel shirt
16·0	61	,,
11·0	44	,,
10·0	83	Thin cotton shirt
8·5	35	,,
17·0	97	,,
4·6	33	,,
11·0	58	,,
7·7	43	,,
10·0	47	,,
6·0	46	,,
7·0	41	,,
5·0	31	,,
4·0	28	Thin cotton shirt and drill jacket
4·0	32	,, ,,
2·0	20	,, ,,
2·0	16	,, ,,
4·3	26	,, ,,
4·0	23	,, ,,
3·2	19	,, ,,
4·5	38	,, ,,

The following exhibits the effect of a fan blowing on the body in a chamber in conditions of tropical moist heat. The rate of loss of heat by a wet kata lying between the shirt and the skin is almost trebled by the fan.

Room temperature—95° F. dry ; 83° F. wet bulb.
Cooling rate between skin and shirt.

	Wet kata 110-100° F.[1]	Dry kata 110-100° F.
Without electric fan .	229 sec.	855 sec.
With electric fan blowing on to body . . .	84 ,,	454 ,,

There is an immense disadvantage in tropical heat in clothes which lessen evaporation. The ideal protection of a white in the tropics from the sun is an umbrella allowing free movement of air, and sweating. The nearly naked native seeks the shade as a monkey does, or wears an umbrella-like hat. The dress in the tropics should cover as little of the body as possible, and where covering is deemed necessary by fashion or custom, the materials used should allow the freest ventilation possible. The thighs and arms swinging in walking pump air in and out of loose shorts and sleeves, a fanning action. The Egyptian robes are cool garments provided little underwear is added. As the native walks his garments sway and flap in ungainly fashion, but in doing so cause air currents which have a cooling effect. H. E. Roaf concludes that a porous substitute for puttees and boots is required.

[1] Calibrated thus.

Each respiratory or other movement tends to produce currents of air in and out of the clothing. One has merely to watch men marching in shorts to see that the movement of the legs inside the trousers pumps air in and out the whole length of the thigh. 'I have been told', wrote Roaf, '(I cannot speak from experience as we are all in service uniform) that a stiff shirt is really as cool, or cooler, than a soft shirt. This may be due to a bellows action of the shirt with each respiratory movement.'

Roaf finds loose night-gowns with loose sleeves are cooler than pyjamas. 'I have designed', he writes, 'some pyjamas which allow the maximum amount of ventilation whilst covering everything required for modesty. They consist of a front piece extended as far down as the knees, united to the back over the shoulders and at the sides by tapes. The back consists of an upper portion loose and a lower portion like drawers fitting in at the waist. The whole is made very loose so that the air blows in through the leg-holes (knee level), the sides, the top, and the middle of the back. For cold weather I think that something of the nature of what I have labelled composition would be useful. Several very thin layers are relatively warmer, weigh less, and impede movement less, than one thick layer. My composition consists of an inside layer of Japanese crêpe, two layers of mosquito netting (or butter muslin), and an outer layer of China silk. It is practically as warm as serge, is two-thirds of its weight, and much more pliable. Such a garment will be lighter than wool, will not alter on washing, and will be less impeding to movement. The outer and inner layers will protect the delicate middle layers from wearing or tearing.'

For the guidance of C.O.'s and M.O.'s who have not experienced campaigning in hot climates, H. E. Roaf drew up the following suggestions:

When the air temperature approaches that of the body, heat loss is entirely due to evaporation, and in order to promote evaporation one must increase the circulation of air in contact with the body.

All parades between reveille and retreat, from May to September (including guards), should be in shirt-sleeves. Shorts should be worn on all occasions, even when walking out.

The shirts should be open at the neck, and a smart appearance can be given to the shirt by removing the neck-band and a V-shaped interval in front and neatly edging the hole.

Rolled-up sleeves prevent the air from blowing through the arm-holes, so the sleeves should be cut off between the elbow and shoulder and edged. This enables the air to blow right across the chest from arm-hole to arm-hole. Very much the same effect can be obtained, and the arms retained for warmth when resting at night, by splitting the arm-seam up above the elbow and adding an extra button just below the elbow. A sleeve thus treated will roll up, leaving a space for air to blow up between it and the arm, yet it can be unrolled and buttoned up to keep the arm warm when so required.

For heavy work in hot weather the shirt may be split below the armpit to allow ventilation, and if provided with a button the slit can be opened or closed as required.

For marching, the shirt may be pulled outside the trousers: this keeps the men much cooler, and the lower end of the shirt keeps dry, so

that, when the march is over, the dry shirt end may be tucked in and thus prevent chilling of the abdomen.

For warm days and cold nights shorts may be provided with cuffs, so that they can be turned up during the day and turned down at night to keep the thighs warm.

Stocking puttees, or canvas gaiters, are better than puttees for hot weather.

How to get over an absurd etiquette is illustrated by the following note: 'In places like Cairo, where one is not allowed to go about

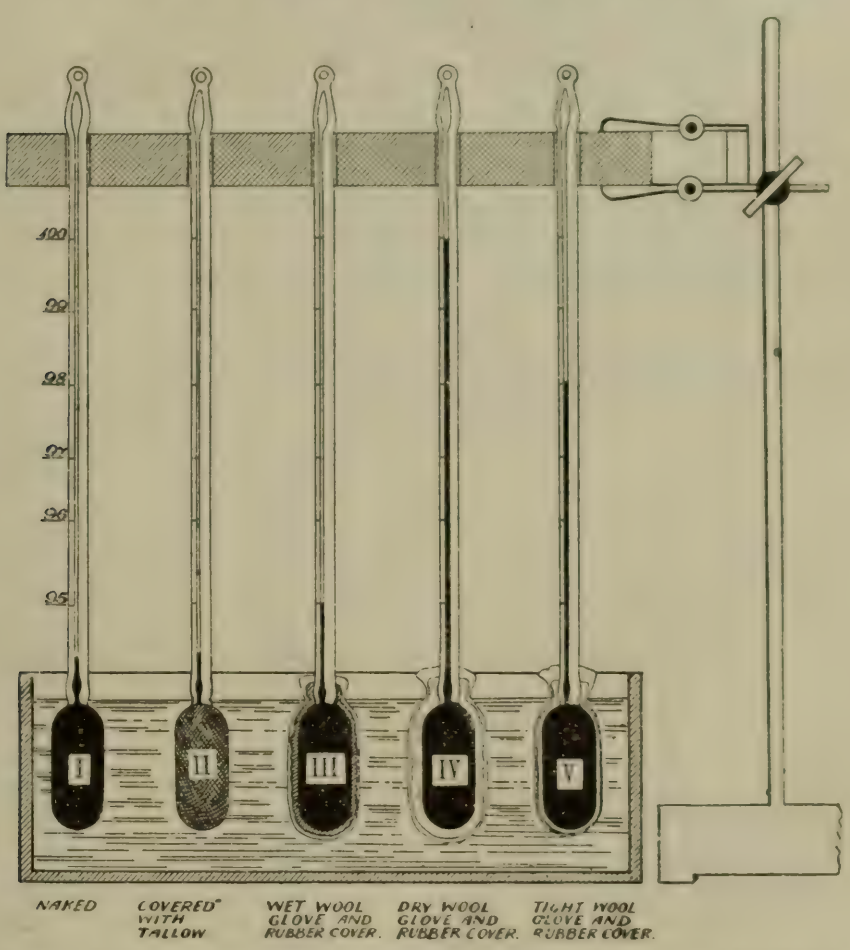


Fig. 38. Kata-thermometers naked and clothed; warmed up together in hot water. On substituting cold water the cooling rates are shown by projecting the menisci on to a screen.

in shirt-sleeves, I have made for myself a collar and a small extension (dicky) which clips on inside of my drill jacket. That with a tie looks like a complete outfit, but I wear only a jacket without a shirt. No one notices the difference, but I think I feel cooler. The jacket is fairly loose and allows a certain amount of ventilation. As the jackets are frequently washed there is no need for underwear.'

For sleeping, in place of a mattress a hammock or bed-floor made of canvas or tape should be used. The quantity of bedding above and below should be the same so that the sleeper is equally cooled all over by a draught.

The next table shows the protection from the great cooling power of water afforded by dry and damp clothing. A kata-thermometer,

double the ordinary size, was used, and cooling timed from 110°–90° F.

One thick wool glove and a thin waterproof rubber cover made the cooling period 12–13 times longer; two thick wool gloves and a thin rubber cover, 18 times. A thick layer of vaseline has practically no effect in slowing the cooling of the kata. It acts on the skin by preventing water soaking into the skin. The dry skin has a much lower conductivity than the wet skin. This is why the naked Fuegians grease themselves with fish oil, channel swimmers with lanoline, and why Italian mountain soldiers wrap their feet in linen covered with an antiseptic ointment. A thick wool garment protects somewhat, even though wet through; those immersed in the sea through shipwreck and waiting to be picked up should therefore keep their clothes on and so conserve their body heat; the more air entangled and confined under the clothes the better. A waterproof cover and thick woollens beneath, such as the diver wears, or the floating suits which were supplied to the American soldiers crossing the Atlantic in peril of submarine attack, afford a very complete protection.

TABLE XXV.—*Large Kata-thermometer in Water at* 11·5° *C.*

	Cooling period 110–90° F.	Cooling period prolonged
Naked	33½ sec.	
Wet wool glove	46	1·3 times
Damp wool glove and rubber cover	151½	4·5
Dry wool glove over damp one and rubber cover	248	7·4
Water at 10° *C.*		
Naked	30 sec.	
Wet wool glove and rubber cover	96	3·2 times
Two damp wool gloves and rubber cover	130	4·3
Wool glove and rubber cover	408	13·6
Two wool gloves and rubber cover	556	18·5
Water at 11·5° *C.*		
Naked	33½ sec.	
Thick wool glove wet	47	1·4 times
Damp wool glove and thin rubber cover	151½	4·5
Damp wool glove, dry glove over this, and thin rubber cover	248	7·4
Dry wool glove and rubber cover	433	12·9
Water at 12–13° *C.*		
Naked	37 sec.	
Damp wool and rubber cover	158	4·2 times
Damp wool and dry suede and rubber cover	252	7·0
Dry wool and rubber cover	391	10·5

The next table demonstrates that the heat-retaining power of the clothes in water is due to the entangled and immobilized air.

TABLE XXVI.—*Temperature of Water* 11°–11·5° *C. Large Kata.*

	Cooling period 100–95° F.
Thick woollen glove, loosely put on, and thin rubber cover .	179 sec.
Ditto, moderately loose	116½
Ditto, put on so as to fit tightly	70
Ditto, damp and put on tightly	28

The next table shows observations taken with the bulb of the ordinary kata in water in a thermos flask; the water was not stirred during the observations. The kata was clothed in each case and heated up with the clothes on in front of a fire, so that the clothes were not disturbed during each series of observations, for it was found that graphs obtained when the same clothes were put on and off the kata between each observation were not coincident, the rate of cooling depending upon the amount of still air enclosed by the rubber skin as well as in the meshes of the clothing. The results show that a garment protected by waterproof skin, and put on so as to contain a normal amount of air (not tight fitting) is inferior in a bath of water to an environment of still air. The conduction of the garment tissue itself is very much greater than of dry air, and the tissue, by touching the kata and the rubber skin, conducts heat directly to the water.

TABLE XXVII.—*Cooling Rates of Naked and Clothed Kata in Water.*

Mean temp. of bath	Dry kata cooling power	
29·9° C.	3·5	Knitted wool and rubber skin
23·9	6·8	,, ,,
18·1	9·8	,, ,,
12·0	13·2	,, ,,
29·0	5·9	Chamois leather and rubber skin
23·5	11·1	,, ,,
18·2	15·3	,, ,,
12·5	21·2	,, ,,
5·8	28·2	,, ,,
29·2	5·5	Woven cotton and rubber skin
24·0	11·9	,, ,,
18·0	15·4	,, ,,
12·2	23·9	,, ,,
6·5	28·2	,, ,,
31·9	14·2	Unclothed in water, not stirred
24·5	44·7	,, ,,
18·5	69·4	,, ,,
12·6	90·3	,, ,,
5·5	116·8	,, ,,
Temp. of air		
30·0	1·7	Unclothed in still air
24·0	3·4	,, ,,
18·0	5·0	,, ,,
12·0	6·6	,, ,,
5·0	8·5	,, ,,

A thick knitted wool glove is a better protector than either woven cotton or chamois leather. It contains more air and touches less.

The cooling power of the unstirred water bath is 14 times greater than that of still air at the same temperatures.

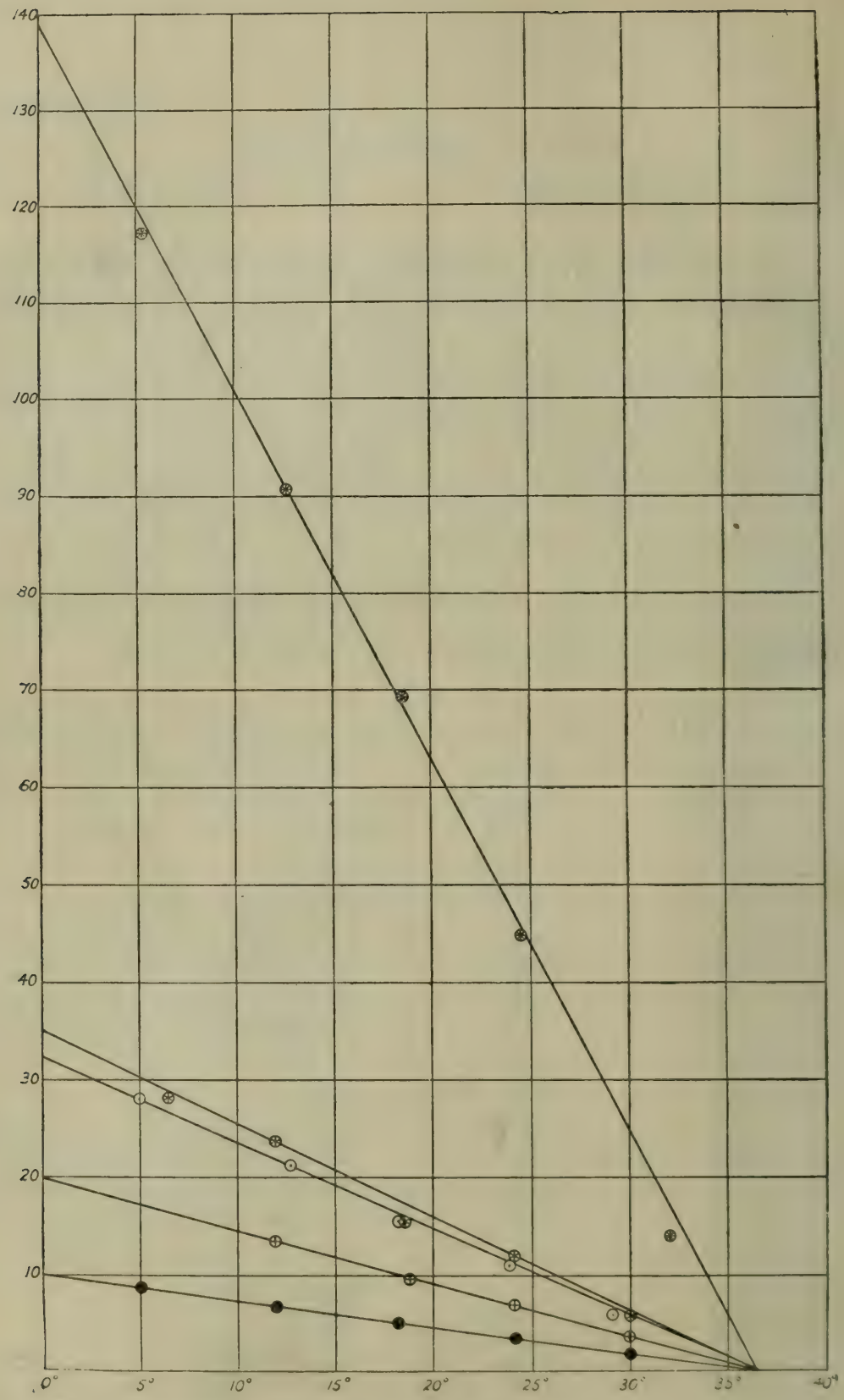


Fig. 39. Cooling powers of: (1) Naked Kata in Still Air.
" " (2) " " Water.
" " (3) Clothed Kata in Water, Wool, and Rubber Skin.
" " (4) " " Suède Skin.
" " (5) " " Woven Cotton Skin.

Trench Feet.

In the first winter of the war the troops were exposed to terrible conditions of cold and wet in cramped positions, resulting in the production of trench foot.[1] The conditions in the first battle of Ypres are described by an officer who wrote:

'The damp has got into my pockets; I am wet from head to heel. My hands are caked in mud. I am wet through, and have nowhere a chance to dry myself. Everything and every pocket is ruined and my money is nothing but a lump of coloured paper. I have tried to dry the lead pencil I am writing with by candlelight in my dug-out, but it is of no use. The water is trickling down the walls and giving me a shower bath all the time. My breeches are thick with mud. I don't suppose even my mother would recognize me at this minute. I have tried in vain to dry my hands. I have blown on them and held them round the candle, but it is no good. My revolver case has turned into putty, and my muffler is more like a mud-pie than anything else. The paper I am writing on I found round some chocolate in my dug-out. Somehow it had kept dry. My watch has stopped, as the water and mud have penetrated it. I have lived on chocolate all day long, and I am going into a cellar near here to get something to eat whenever I can be relieved. The cellar belongs to a ruined farm that has not a wall standing.

'I have just waded through our trenches which are falling to pieces and are filled with mud and water, parapets falling down and all the dug-outs collapsing; the men stand shivering in the bitter wind on sentry duty, others huddled together trying to keep warm, but wet and grimy and caked in mud. Am I a human being? I ask myself. I am gradually getting cold and chilled all through, and will, to keep circulation going, be forced to go round my sentries to see if they are all standing by. We have not slept for nights, and it is hard for everyone. I am cheerful and have a laugh ready for all my men. They have been good to me, and it is marvellous how good they are and how little grumbling there is. I dug one of my men out of the mud yesterday. I thought we had lost him, as it took 1½ hours to dig him out, but I am glad to say we pulled him round and he is quite fit again. Last night I fell into a trench full of water, and the men kept a little box full of wood burning near me all night long and brought me hot tea. They are fine and have worked splendidly all the time.'

The symptoms of trench feet are described thus:

'Gangrene necessitating amputation occasionally occurs; but discoloration of the skin and haemorrhages into it are frequent. The hallux has been more often affected than the other toes; the other regions affected are the heels and the pressure points of the base of the hallux and the ball of the little toe. Oedema is often seen; it is greatest where the tissues are lax and is most noteworthy on the dorsum of the foot. Massive peeling and desquamation in flakes are frequent. The nails often show haemorrhages beneath them. When a nail is pressed upon by the neighbouring toe, haemorrhage may only

[1] For the literature on the subject see Medical Bulletin published by the American Red Cross Society, Paris, February 1918.

occur in the part subjected to the pressure. Pigmentation is sometimes found under pressure points.

'The extensor tendons of the toes may be taut and the hallux intensely dorsiflexed. Active movements of the toes are frequently limited in range and defective in power. The joints and tendon sheaves are not affected. The knee-jerks, ankle-jerks, and the plantar reflexes are always obtainable; occasionally, however, in patients showing a loss of sensibility there may be some difficulty in eliciting the plantar responses.

'Spontaneous pains are frequent in the feet and ankles and may occur in the lower portions of the legs; rarely do they extend above the knee. They are described as "shooting", "gnawing", and "aching", and are said to be constantly present but intensified by movement; yet, when the patient's attention is diverted, he will talk freely and appear at ease. Numbness of the skin and coldness or deadness of the feet are among the frequent symptoms, and many patients describe their sensations as "like walking on their heels".

'An alteration in sensibility is common. The alteration may be an excess of reaction to stimuli, a defect of reaction, or may even amount to total anaesthesia.'[1]

It was agreed that prolonged inactivity of the feet, particularly in the dependent position, and exposure to humid cold were the causes of trench feet. Compression of shoes and puttees was only a secondary factor and often absent. The crouching, half-asleep position of soldiers in the trenches favoured the onset. Officers who were obliged to change their positions frequently were rarely affected. The sitting posture maintained on the fire-step during rest periods tended to make the feet 'go to sleep'. The position impeded the circulation. The arms were affected in the case of wounded who had lain for hours with an arm in water.

Every effort had to be made to keep trenches dry, to shorten shifts in trenches known to be damp, to encourage activity, e.g. stamping and moving toes when standing, waving feet in air when lying down, to supply plenty of food and warm drink, to change and dry socks, wash, massage, and grease the feet in rest cantonments, to keep the boots well greased.

The following letter shows the difficulty there was in carrying out the prophylactic measures in the Ypres salient:

'From the hour we enter the trenches till we get back to billets, the troops will never stand on a dry bit of ground. They will be struggling either in sticky porridge-like mud or plunging through pools of muddy water, sometimes waist-deep. No ordinary boots will keep out the damp, and though for some obscure reason we don't get pneumonia, we cannot help getting our feet into the condition of a washerwoman's hands. This is especially the case when trenches become so bad that long boots of rubber cloth—known as "gum-boots"—have to be issued. These boots are absolutely invaluable. They protect right up to the thighs. But unfortunately they produce a very bad effect on the feet if they are not taken off at least once a day. The skin crinkles and grows soft because there is no proper evaporation of sweat. Also the heel chafes because it is impossible to

[1] E. G. Fearnsides and M. Culpin, *Brit. M. J.*, Jan. 15, 1915.

get an absolute fit. Men must be taught to wear two pairs of socks, and to change them round, inside and out, every day.

'The M.O. is responsible for impressing all these points upon platoon officers. He must also teach and enforce the use of frost-bite grease on feet, socks, and boots, and must advise on arrangements by which sodden socks can be regularly dried either over braziers in the trenches, or on a grand scale near battalion headquarters. It is no easy task to give effect to orders laid down on these subjects, simply because men in the trenches have no place in which they can take off their boots in any comfort. There is nothing to sit on except the floors of the dug-outs, and these are generally muddy, if not actually under water. The roofs mostly leak, and there is no light to speak of. For my own part any undressing in the trenches is a nuisance. I much prefer to endure the discomfort of being soaked from the waist downwards until we go back into billets.'

Baratte prevented trench feet by having all the shoes dipped in warm grease, and by ordering the men to unlace their shoes for a quarter of an hour twice every day.

The Italian Alpine troops used the following preparation:

Melt at the temperature of 50° C.:

93 kg. of pure tallow;
4 kg. „ lard.

Add an emulsion made of:

250 grm. of salicylic acid.
750 „ of aspic essence.
750 „ of oil of lemon.
1,250 „ of lard.

Pieces of linen (medium texture) measuring cm. 45 × 45 were immersed in the liquid, taken out as soon as soaked, put to dry on lines, afterwards folded and enclosed in parchment paper. Such pieces of linen were wrapped round the feet. The essential oil acted as an antiseptic, and this action and the grease together prevented the skin becoming water-logged, inhibiting the growth of bacteria and the formation of the toxic products of their growth, such as ammonium sulphide, out of the keratin of the epidermic scales. These products damage the water-logged tissues and increase the exudation and set up necrotic changes. Fishermen and sailors, whose boots are filled with sea-water, do not suffer, no doubt owing to the salt in the water.

Delepine, by experiments performed on himself, proved that while a thick wool covering when wet somewhat retards the loss of heat in water, but is not enough to prevent 'frigorism', a thin layer of dry air suffices if secured between the skin and the water by means of a waterproof cover.[1] The kata readings given above show the enormous cooling power of water and the protection afforded by dry air entangled in a woollen garment protected by a waterproof outer cover.

'The essential change in trench feet consists in damage of the blood-vessel walls, evidenced by the swelling of the endothelial cells in

[1] *Brit. M. J.*, 1915, ii. 890.

the vessels of all kinds and by the vacuolation of the muscle fibres in the arterial walls. Accompanying these vascular changes there is damage to the cells of the tissues, the whole resulting in excessive exudation of fluid and formation of fibrin in the fluid. Haemorrhages may occur. The lymph channels are distended with a fluid richer in

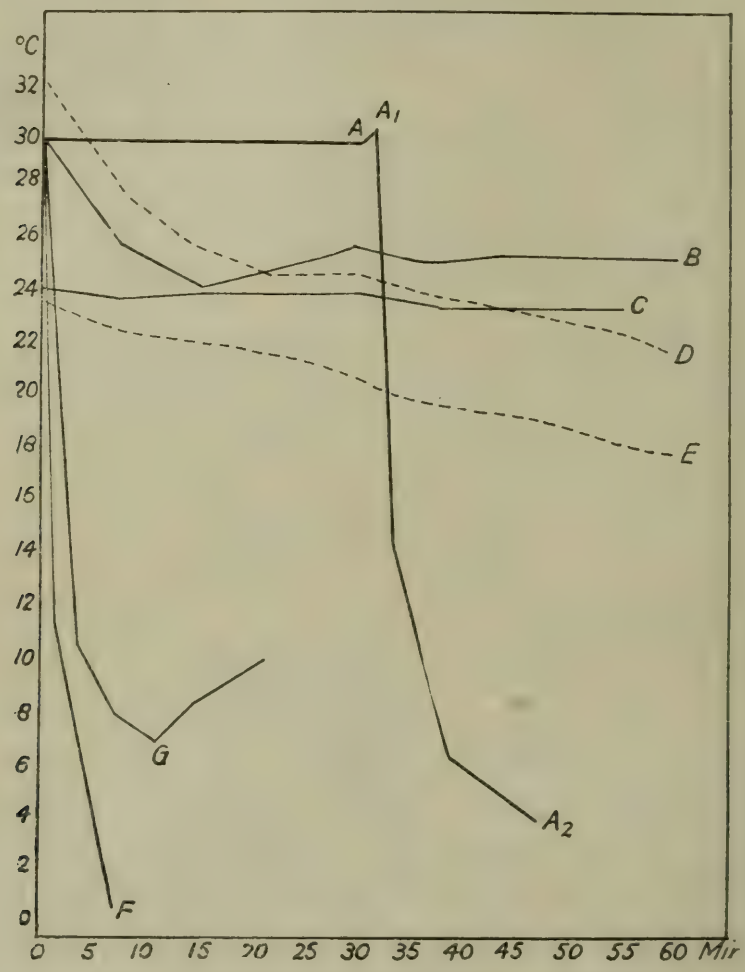


FIG. 40. Skin temperature of feet. Delepine.

A. Hand protected by dry sock and oil-silk bag.

A_1. Water at 35° C. poured into the oil-silk bag so as to wet thoroughly the sock. Very slight rise of skin temperature followed by a very rapid fall A_2. (Compare with F and G.)

B. Warm foot protected by dry sock and oil-silk bag.

C. Cold foot protected by dry sock and oil-silk bag.

D. Warm foot protected by damp and partly wet sock and oil-silk bag.

E. Cold foot protected by damp and partly wet sock and oil-silk bag.

F. Foot covered only with woollen sock and plunged into refrigerating mixture.

G. Hand unprotected plunged into double its bulk of water at 0° C. (For comparison with F and A_1 to A_2.)

protein than normal. By the lethal changes an osmotic pressure is created which shuts off the blood-supply. The necrobiotic changes affect the muscles, the nerves are more resistant, but there are signs of oedema of the axis cylinders. The connective tissue fibres become swollen with fluid and disintegrated.'[1]

The tissue changes are comparable to those produced by other

[1] Lorraine Smith, Ritchie, and Dawson, *J. Path. and Bacteriol.*, 1915, **20**, 159.

agents which damage the vitality of the cells, e.g. a chemical irritant or bacterial poison.

N. C. Lake [1] found that, while a temperature of −6° C. is necessary to produce gross destruction of cells and freezing of blood, tissues cooled to about 15° C. have their metabolism seriously interfered with. There results in a frost-bitten part a rapid exudation of fluid and blood, clotting of the exudation, and shutting off of the blood-supply. For the actual frost-bitten part nothing can be done; treatment must be directed to prevent vascular congestion on thawing and consequent oedema, and swelling and obstruction of the circulation in the adjoining parts.

Heat applied during the period of restoration of the circulation tends to favour the production of necrosis. The feet require to be kept in the elevated position, uncovered, free to move and cool, so that the vessels are not congested and the oedema drained away.

The tissues of cold-blooded animals withstand a much greater degree of cooling.

A frog or fish may be frozen rigid and survive on thawing, but only the intra-cellular liquids, not the cells, may be frozen. The latter may be protected by concentration of contents and the oxidative process, and by the extra-cellular ice formation.[2]

Frog's heart-muscle survives a temperature of −2·5° C. but is killed by a temperature of −3·0°; peripheral nerves of the frog are not killed by much lower temperatures.

The potato beetle before hibernation loses almost one-third of its weight of water, with consequent concentration and lowering of freezing-point of its protoplasm. Rotifers encapsulate themselves with a slimy secretion which resists extreme desiccation, and so encapsulated they survive freezing. Bacteria survive exposure to the temperature of liquid air.

Hibernating mammals become cold-blooded, but wake up if an attempt is made to freeze them.

Much as it has been discussed, there is, so far, no causal explanation of hibernation of marmots, dormice, bats, &c. It is suggested that the nervous system loses its specific sensitiveness to external cold, and returns to the cold-blooded condition of that of the new-born with the sensitiveness as yet undeveloped.

It has been suggested that something in the food-supply leads to this. Some of these animals hibernate occasionally in summer months, and this fact is difficult to harmonize with any hypothesis.[3]

The hibernation of Indian fakirs considered by Verworn can be left to romantic treatment as in Stevenson's *The Master of Ballantrae.*

[1] *Lancet*, Oct. 13, 1917.
[2] Cf. Cameron and Brownlee, *Quart. J. Exper. Physiol.*, 1913, 7, 115.
[3] Cf. A. T. Rasmussen, 'Theories of Hibernation', *Amer. Nat.*, 1916, 50, 609.

CHAPTER IX

Methods of Ventilation and Heating.

Successful ventilation depends on the prevention of stagnation of body heat on the one hand, and of uncomfortable chilling of the body on the other. Not only should it prevent heat stagnation in schools and workshops, but should stimulate the worker.

Experience shows that in crowded shut-up places, rooms, railway cars, &c., the conditions are comfortable when the temperature is low, so long as there are no cold draughts, and uncomfortable when it is high, regardless of the chemical purity of the air.

The greater the number of air-changes, however, the less is the infection by saliva sprayed out by carriers of catarrhal disease in coughing, sneezing, and speaking.

As an example of how ventilation may lessen infectious catarrhs, Winslow gives the following :

'The installation of a 75-dollar system of ventilation in the operating theatre of the Telephone Exchange, at Cambridge, Mass., was followed by a reduction in winter absences from over 4·5 to 1·9 per cent. of the force employed.'

Chemical purity is of importance in mines and certain factories wherein poisonous gases or fumes arise, and in rooms where products of imperfect combustion escape, e.g. stoves and gas-burners improperly flued.

Gas rings and Bunsen burners and gas 'radiators', if unflued, taint the air as well as heat it. The extreme top of the Bunsen flame is the only portion which can be used for heating a solid without liberating deleterious gases. The flame is at 1,500° C., and a pot of water at 100° C. puts out the flame before combustion is complete. Thus the gases escaping on heating a pot of water (1) half an inch above the inner cone, (2) at the extreme outer tip, of the flame were

	1	2
Nitrogen	75·75	79·17
Water vapour	13·47	14·29
Carbon dioxide	2·99	5·15
Carbon monoxide	3·69	nil
Methane	0·51	0·31
Acetylene	0·04	nil
Hydrogen	3·55	0·47

(Vivian Lewis.)

Anhydrous sulphuric acid (SO_3) is formed from the sulphur in the gas. One part in 500,000 is irritating.

Using a paraffin lamp in a chamber, Haldane says there was no unpleasant quality in the air when the carbon dioxide reached 75 per 10,000. If Oxford town gas were burnt, the air became unpleasant when the carbon dioxide reached 30 to 40 parts per 10,000. The 8 to

9 grains of sulphur per cubic foot of gas is responsible for the difference. The purification of gas from sulphur is then of importance. Ventilation must be ample enough to remove the sulphur acid produced by gas: it will be so if the atmosphere is kept cool.

Modern gas fires properly constructed and flued do not allow the escape of fumes into the room. To test for such escape a plate glass cooled in iced water and dried is held edgewise against the canopy. A deposit of dew indicates escape of flue gases.

It was pointed out that the mere observation of the temperature of the air in a room by an ordinary thermometer is no index of comfort, and gives no measure of the amount of heat in it. The thermometer measures the *degree*, not the quantity of heat, and gives no measure of radiation or of humidity, of drying power or cooling power exerted on the skin.

In stagnant air in a room without source of radiant energy,

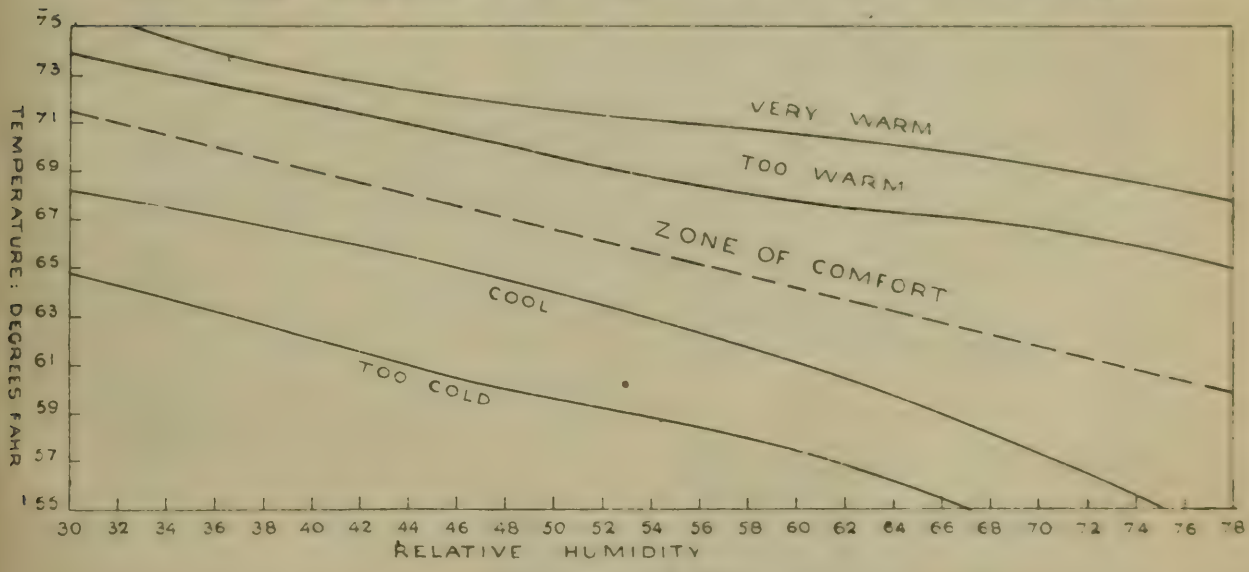


Fig. 41. E. V. Hill.

the cooling power by convection and radiation depends on the temperature—$H = 0{\cdot}27\ \theta$ for the dry kata-thermometer ($\theta = 36{\cdot}5°$ C. —the air temperature). In such rooms, however, the wet bulb is more indicative of comfort than the dry bulb.

In a *stagnant* atmosphere comfort for sedentary workers has, then, been defined in terms of temperature and relative humidity. Low humidity compensates for high temperature as shown by Fig. 41, plotted out by E. V. Hill[1] in an experimental chamber at Chicago. For a sedentary man 56° F. (wet bulb) was found to be most comfortable whatever the dry bulb; 58° was too warm, 52° too cold. The comfort curve for the wet-bulb temperature with air movement is given by Fig. 42, plotted out by the same author.

T. Hough finds there is a neutral region, about 68°–70° F. dry bulb, at which high humidity has little effect on sedentary workers in a room (U.S.A.). At 65° F. high humidity cools, over 70° F. perspiration comes into play to cool the skin, and high humidity lessens this and so heats the skin. If enough cold air were pumped into a crowded room at 68°–70°

[1] Cited after A. H. Barker, *J. Soc. Architects*, 1918, 11, 3.

there was no complaint, while complaints arose so soon as the temperature was allowed to rise above 70°. Two or three degrees made all the difference.

How defective ventilation commonly may be is illustrated by the following: In a hall in Birmingham, with an audience of 3,100 persons, the temperature rose in 3 hours from 13·5° to 27° C. (56½°–81° F.). If the air was still in this hall the dry kata cooling power would have sunk from 6·2° to 2·5°.

In a theatre in Munich, in one hour the temperature rose from

15·2° to 16·5° C. in the orchestra
16·2 „ 19·4 „ first gallery
16·2 „ 25·4 „ second gallery

In such crowded places the wet-bulb temperature is not far behind the dry-bulb, and the vapour tension becomes high and the evaporative power greatly reduced.

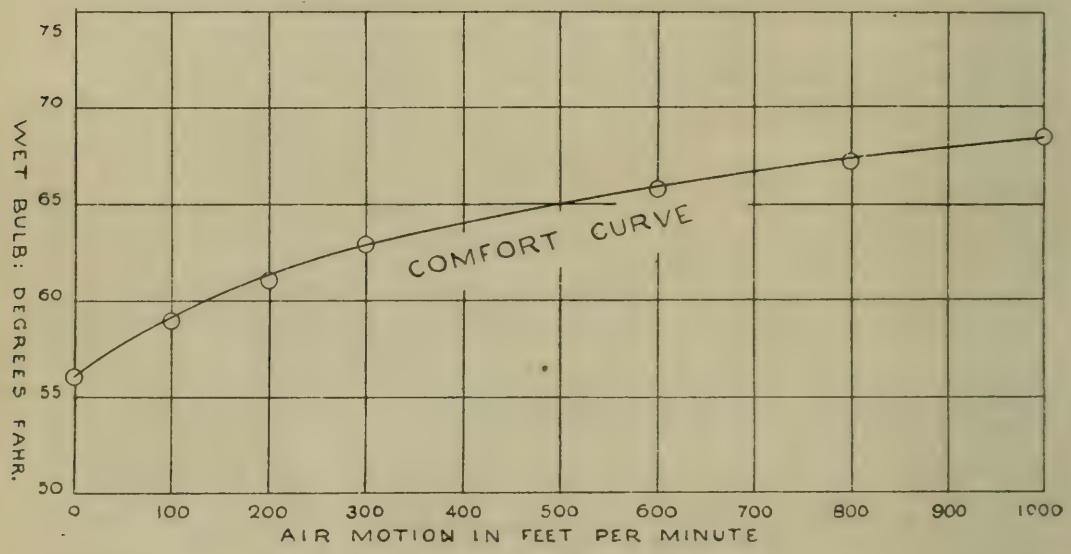


Fig. 42. E. V. Hill.

In 14 cinema theatres ventilated by a certain number of shafts in the roof, the carbon dioxide varied from 28½ to 47 parts per 10,000, the temperature in January from 56° to 65° F., the relative humidity from 59 to 88.[1]

The cubic space varied from 50 to 170 cubic feet per person. Such places would be hot in warm weather, and with such few changes of air they facilitate the spread of infection.

The following are types of observations taken in the Birmingham cinema theatres in January when filled:

A. Three openings in roof, no fans, no special fresh air inlets, gas radiators.

Temperature 57°, CO_2 28·6.

B. Plenum fan 4 feet diam. delivering fresh air to sides of building by 14 openings, arrangement for washing and heating incoming air when necessary. Vacuum fan 4 feet diam. extracting from five large openings in roof.

Temperature 61°–70°, CO_2 8·6–12·8.

[1] J. Robertson, Rep. to Public Health Committee, Birmingham, April 14, 1919.

C. One opening in roof, no fans, three windows fitted with hoppers.

Temperature 63°, CO_2 46·7.

D. Four openings in roof, two extraction fans. Ten small inlets, all closed. Hot-water radiators.

Temperature 66°, CO_2 36.

At an employees' canteen in New York the temperature records varied between 72° and 77° F., half being over 75°, the carbon dioxide ranging from 10 to 20 parts per 10,000.

In the rooms of a large office building in New York C. E. A. Winslow found temperatures of 72°–75° in 34 per cent. of all observations, 76° and over in 9 per cent.

In a University dining hall 28 per cent. of all observations were between 71° and 72°, 16 per cent. 73° and over.

Two window-ventilated hospital wards in New Haven, on the other hand, showed less than 5 per cent. of all records over 70°. In an office with three thermostats in disrepair, the temperature for 21 days in February and March never fell as low as 70° during working hours, and was usually in the neighbourhood of 80°.

Using the kata-thermometer as the measure and the evidence collected in rooms which felt fresh or close, the conclusion is reached that the dry kata cooling power for sedentary workers should not be lower than 6, and the wet 'kata' cooling power lower than 18. Preferable figures for schools and offices are D.K. 7 and W.K. 20–23; higher cooling powers are comfortable if the feet are kept warm.

The following are examples of types of readings selected from many hundreds taken in factories:

Dry kata	Wet kata	Dry bulb	
8·5	26	54·5° F.	Winter day, sedentary workers, too cold for comfort.
6	18–20	63·0	Sedentary workers, comfortable condition.
8–12	22–26	65·0	Cross-circulation with hopper windows on cool summer day, excellent conditions for fairly hard work done in factory;
3·0	13·0	82·5	Warm summer day, serrated roofed one-story factory, enervating conditions, fans required to blow air in on workers.

During the war rooms of the National Gallery were allocated to clerks engaged in auditing accounts. The galleries are high and lit by skylights, ventilated by small roof openings, heated by steam pipes laid in tunnels under the floor, these ventilated by gratings in the walls. There was ample space and no overcrowding. The conditions were such as to give a stagnant monotonous atmosphere, and such readings on close days as dry kata cooling power 4·5, wet kata 16, dry bulb 73, wet bulb 63. In such stagnant air infectious colds were easily spread, the workers sitting close.

The clerks worked from 9 a.m. to 1 p.m., and from 2 to 5.30–6 p.m., at the monotonous and trying occupation of auditing figures. There were no windows and they could get no change of sight, no refreshing breeze. Ventilation was maintained only by convection. The complaints of want of ventilation were great. Clerks said there was no oxygen, they could 'cut the air with a knife'—expressions

provoked by the absence of visible ventilation, and based on the chemical theory of vitiated air. One girl, pale and anaemic, fanning herself, said she would like to tear open the skylights.

The absences of these employees from ill-health were considerable, and the economic waste arising therefrom great.

The chemical purity of this atmosphere could not be assailed; the carbon dioxide increase was insignificant. The hygienic expert called in found no cause for condemnation. The higher official marked the papers 'no action', but the kata readings showed that the air had too low a cooling power and evaporative power. The working hours were too continuous and too long, and the work most monotonous. The girls travelled by warm, shut-up, crowded tube trains, probably they took no active exercise at home.

Girls so employed ought to be exercised every hour or so for five minutes just as a school; they ought to have change of employment, so many hours at the desk and so many at hand-work in a factory, or better in the garden.

The following were taken in a Rand mine where natives were hammer-drilling. In the first case the worker, wearing loin-cloth and trousers, was bathed in perspiration, and lost $4\frac{3}{4}$ lb. of water in five hours by sweating. In the second case there was no visible perspiration, and the native, wearing loin-cloth only, lost 3 lb. of water in five hours. In both cases higher cooling powers are required.

Ventilation is increased in these mines by compressed air and movement of machinery:

Dry kata	Wet kata	Dry bulb	Wet bulb	Cheek temperature
4	15·0	79·5	77·5	35·5
6	16·5	78·0	76·0	33·0

The ventilating effect of machinery was discussed in Part I of this Report. The dry kata cooling power, for example, was, in a machine shop 5 with the machinery stopped and 8 with it started, in a spinning-mill 3 and 5, the wet kata cooling power being 13 and 19, and 27 near a driving-wheel.

In a factory where the operatives were 'fed-up', and frequently threw up their job after being trained in it, I found dry kata readings of $3\frac{1}{2}$–5 and wet kata readings of 13–15 on a cool day. In summer weather in the factory the conditions must be very trying. The factory was a most 'up-to-date' one-storey building, but fitted with most defective ventilation apparatus, and having many hot processes going on inside it. Fig. 43 shows how an air trunk in this factory discharged air at the distal end, and sucked air in through the ventilating slots near the blower fan. Fig. 44 shows that with a blower fan at either end of the trunk, air was discharged from the slots only in the middle of the trunk and sucked in at either end. In both cases the ventilation was most defective. Far better ventilation was obtained by arranging each blower to blow through a single wide narrow slot placed at a height just above the heads of the workers.

The readings in an ill-ventilated foundry were:

Dry kata	Wet kata	Dry bulb	Wet bulb	
1·4	8·1	89	72	forge end
1·7	12·2	103	72	furnace end

It was pointed out in Part I of this Report, p. 55, that by means of a fan a man can escape from under the curse 'in the sweat of thy face shalt thou eat bread', and that it will pay the employer to so regulate the cooling power as to prevent heat-stagnation and sweating, and, while not uncomfortably cooling the worker, to stimulate him by

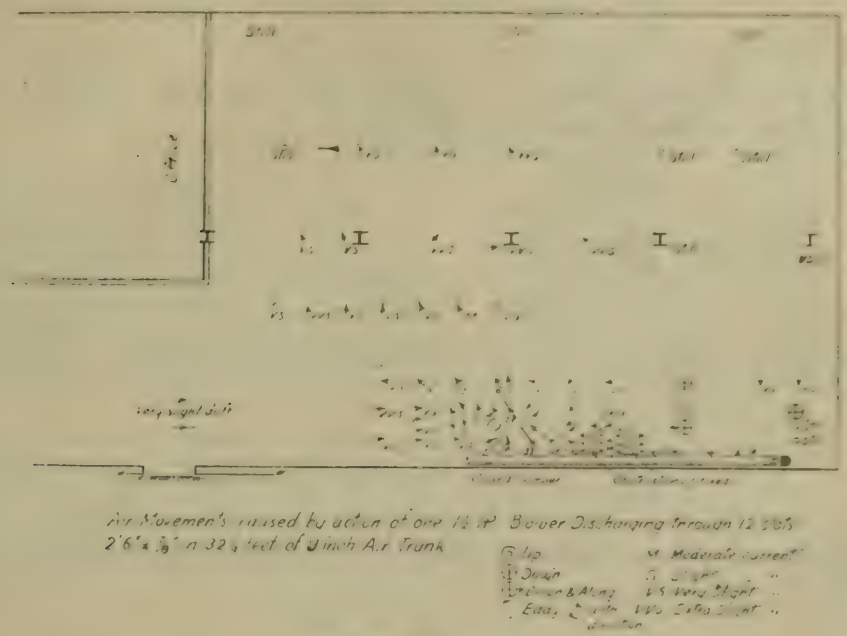


Fig. 43. T. Angus.

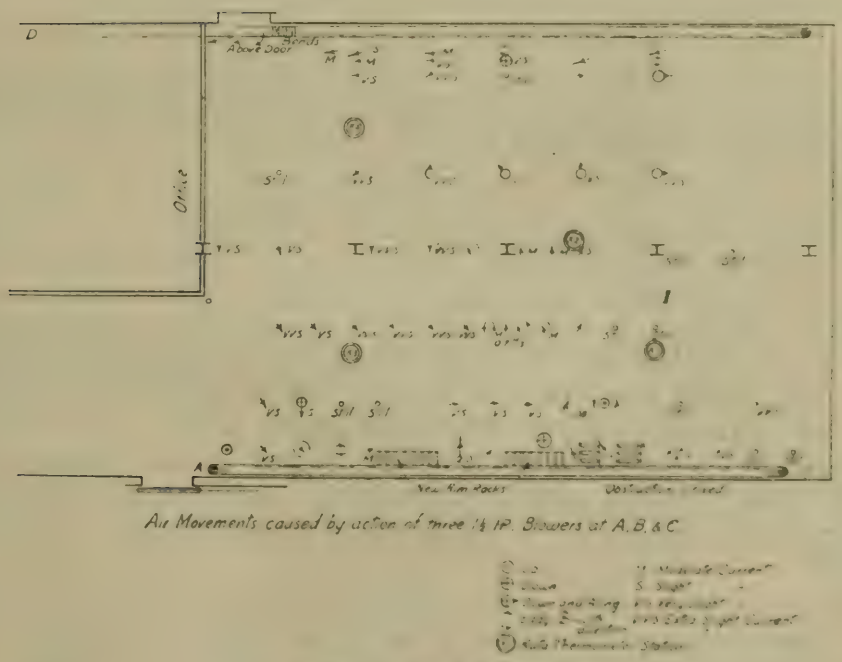


Fig. 44. T. Angus.

sufficient movement of the air, and so secure greatest efficiency and least fatigue and ill-health. To secure this stimulation, fans are required for warm weather, when window ventilation fails, in all workplaces: for both winter and summer use in workshops where hard physical work is done. The following table sets forth suggestions as to the movement of air in feet per minute to obtain cooling powers suitable for various occupations.

Dry kata cooling power 6, suitable for sedentary workers, clerks, tailors, &c.:

	Ft. per min.	At temperature ° F.
Less than	1	60
	3	65
	12	70
	40	75

Dry kata cooling power 7, suitable for very light physical work, e.g. machine-minders, weavers, spinners:

1	55
4	60
12	65
30	70
70	75

Dry kata cooling power 8, suitable for light physical work:

4	55
12	60
25	65
50	70

Dry kata cooling power 9, suitable for moderate physical work, carpenters, metal workers:

10	55
20	60
40	65
80	70

Dry kata cooling power 10, suitable for moderate heavy physical work, carpenters, metal workers:

20	55
40	60
70	65
100	70

From E. P. Cathcart's[1] measurements of metabolism I calculate a man wants a dry kata cooling power of 5–6 at meals, 7 cleaning uniform, 8–9 cleaning windows, 10–11 scrubbing a floor, 11 at drill, 14 walking 3 miles an hour; from A. D. Waller's[2] measurements that a proof reader wants a cooling power of 4, a compositor 6–7, a worker in a printing foundry 9, a tailor $6\frac{1}{2}$, a dock labourer doing piecework 13.

The heat given off by a man walking 5 miles an hour is estimated to be equal to that given off by 21 sq. ft. of radiator surface at a temperature about 100° F. above the air temperature (A. H. Barker).

Heavy work requires to be done in open sheds or out of doors, or with cold air sprays under the control of the individual worker. These sprays are also of great value in hot occupations, e.g. glass blowers, furnace men.

[1] *Proc. Physiol. Soc.*, Jan. 31, 1920. Energy Expenditure of Infantry Recruit, H.M. Stat. Office, 1919.

[2] *Proc. Physiol. Soc.*, Dec. 20, 1919; Jan. 31, 1920.

The velocity of wind required to give a dry kata cooling power of 10 is 0·5 metres per sec. at 20·3° C. (68½° F.). Supposing the temperature were 10° C. (50° F.), then the mean temperature of the cooling kata surface would have to be 26·2° C. in place of 36·5° C. to keep the cooling power still at 10—a cool wind constricts the vessels of the skin and keeps the surface of the skin cool.

The cubic space per person under various conditions is illustrated by the following:

	cu. ft.
Poor law infirmaries, according to regulation	1,000
Soldier's ,, ,,	600
Pauper's dormitories ,,	300
Sick bay, navy	250
Average mess deck, navy	150
Average cinema theatres, Birmingham	140
Sleeping deck, 'Dreadnought' class	86
Soldier's military transport	80
Sleeping deck, 'Formidable' class	68
A crowded dug-out curtained against gas	3

The changes of air per hour in a room occupied by people can be determined by estimating the carbon dioxide in the air.

The air supply is inversely as the increase of CO_2 due to respiration. It may be computed from the following equation (T. Crowder):[1]

$$A = \frac{vp}{x - N},$$

where v = the CO_2 produced by one person (cubic feet per hour),
p = the number of people in the room,
x = the proportion of CO_2 found in the air of the room,
N = the proportion of CO_2 in the outside air,
and A = the air supply to the room (cubic feet per hour).

It is seen that vp is the CO_2 produced by the occupants and $x - N$ is the respiratory contamination. We may substitute known values for the letters v and N in the above equation. The amount of CO_2 produced by one person will average very close to 0·6 cubic feet per hour, and the average proportion of CO_2 in the air 4 parts in 10,000. Making the substitution the equation becomes

$$A = \frac{0{\cdot}6\,p}{x - 0{\cdot}0004}.$$

Completing the computation of air supply for various assumed proportions of CO_2 the results would be, when the space is occupied by one person only, i.e. when p is the unit 1, as follows:

CO_2 = 0·0020 (resp. contam. = 0·0016), hourly air supply = 375 cu. ft.
CO_2 = 0·0016 (resp. contam. = 0·0012), hourly air supply = 500 cu. ft.
CO_2 = 0·0012 (resp. contam. = 0·0008), hourly air supply = 750 cu. ft.
CO_2 = 0·0010 (resp. contam. = 0·0006), hourly air supply = 1,000 cu. ft.
CO_2 = 0·0009 (resp. contam. = 0·0005), hourly air supply = 1,200 cu. ft.
CO_2 = 0·0008 (resp. contam. = 0·0004), hourly air supply = 1,500 cu. ft.
CO_2 = 0·0007 (resp. contam. = 0·0003), hourly air supply = 2,000 cu. ft.
CO_2 = 0·0006 (resp. contam. = 0·0002), hourly air supply = 3,000 cu. ft.
CO_2 = 0·0005 (resp. contam. = 0·0001), hourly air supply = 6,000 cu. ft.

These same figures apply to a room with any number of occupants

[1] *Am. Pub. Health Ass. Rep.*, Sept. 1910, and *Arch. Int. Med.*, 1913, **11**, 66.

provided we add ' per person ' after ' hourly air supply '. In order for the air to maintain a constant respiratory contamination the volume supplied must be multiplied as often as we multiply the occupants—the air supply must maintain a constant ratio to the CO_2 produced. Each person will contaminate 1,000 cubic feet of air per hour to the extent of 6 parts of CO_2 in 10,000 of air (and the total CO_2 will be 10 parts in 10,000). One person will need simply the 1,000 cubic feet per hour if the contamination is to be held at this level; fifty will need 1,000 cubic feet each, or 50,000 cubic feet.

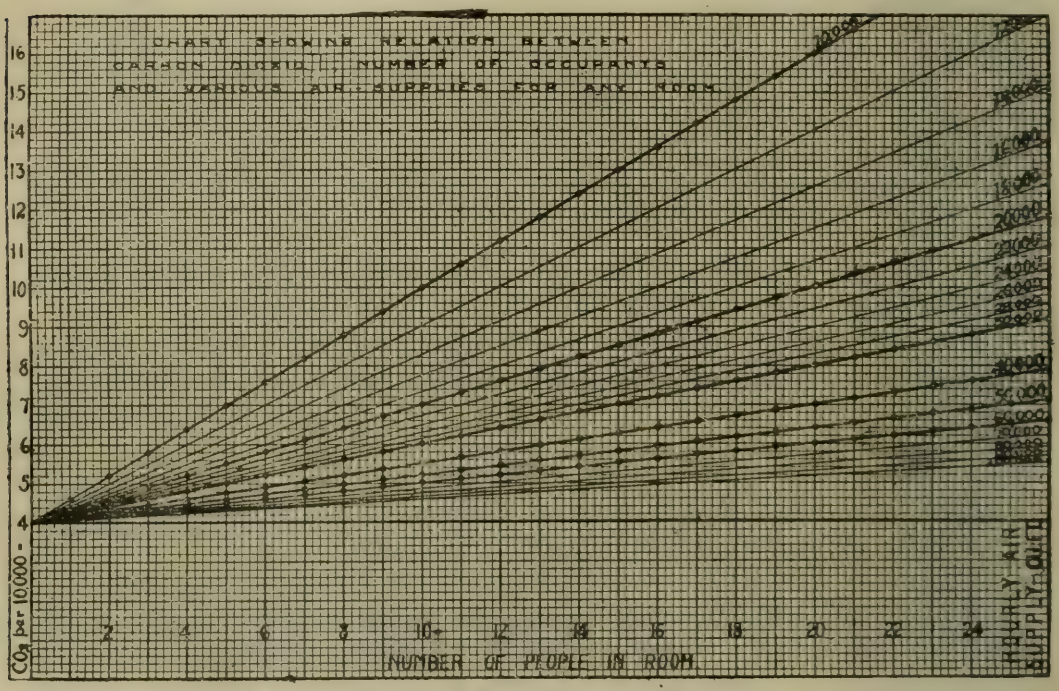


Fig. 45. T. Crowder.

The following observations were recorded in U.S.A. by Crowder:[1]

	No. of observations	CO_2 per 10,000	Calculated air supply per person per hour in cu. ft.
Street cars	45	15-29	541
Elevated cars	17	14-26½	674
Suburban coaches	47	14-38	583
Stores	23	9-10	1,250
Restaurants	51	16-26	496
Offices	26	14-19	670

So long as there is a temperature difference between the air inside and outside a room, and when the wind is blowing, natural ventilation takes place through every chink and cranny, and to a far larger extent than is usually recognized. Thus Crowder[2] investigated by the CO_2 method the ventilation of railway sleeping cars when running. It was for wood cars with window sashes in the roof:

Fully closed	18,500 cu. ft. per hour
Open roof sashes	28,300
Open roof sashes and end doors	40,700

[1] *Arch. Int. Med.*, 1911, **7**, 85. [2] Ib., 1913, **11**, 66.

Steel cars. Exhaust ventilation:

Fully closed	53,000 cu. ft. per hour
Drop sashes in end doors, open . .	65,900

The steel sleeping cars have six to eight exhaust ventilators[1] fitted in the roof actuated by the motion of the train. The cars are lighted

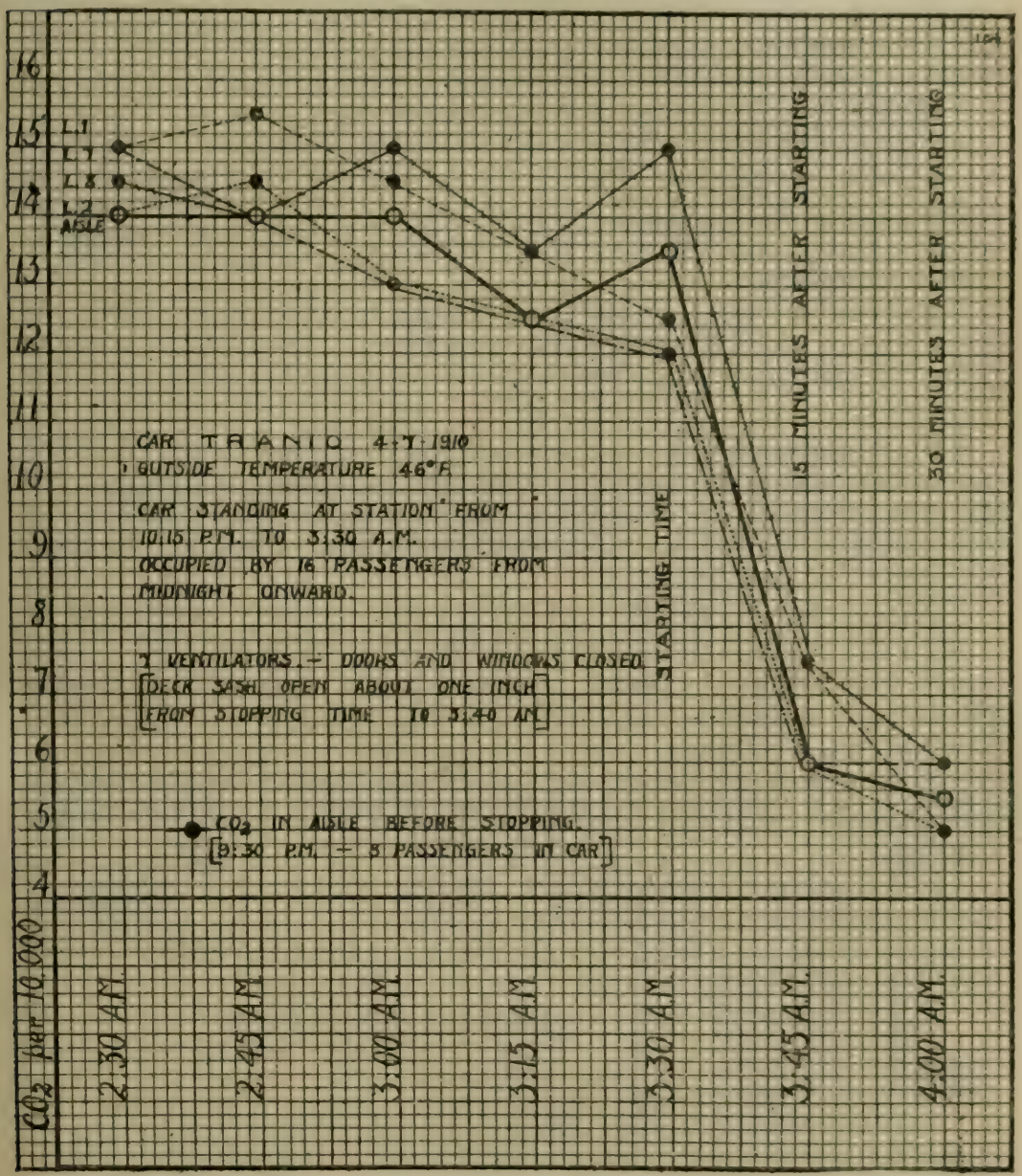


FIG. 46. Pullman car with exhaust ventilators in roof. Curve of CO_2 percentage. T. Crowder.

by electricity, and the upper portion is made almost entirely free from natural openings, so as to prevent short circuiting of the ventilation. The air enters by the crevices of windows, doors, &c., in the breathing zone.

Figure 46 shows the effect of starting a train on the ventilation,

[1] Garland ventilator, *Railway Age*, 1906, **41**, 847.

by exhaust ventilators fixed to the roof, of a sleeping car. Extract fans removing the same volume of air would have a similar effect on the stationary car.

Crowder found in the case of the cars that with the exhaust method the air supply is always adequate to prevent monotony and stagnation of the conditions surrounding the skin. Yet the streams of incoming cold air are individually of such small volume, and take on such irregular and diffuse movement into the stiller body of air within, that the discomfort of draughts is almost entirely avoided.

'Experience has shown that it is necessary to have in American sleeping cars at least twice as much radiating surface as is demanded in common practice for heating the same space in houses: this in order to meet rapidly changing conditions and warm the large volume of air received and discharged, so that it will maintain comfort to inactive passengers.'

Railway carriages should have the steam pipes placed under the floor so as to warm the feet of the passengers. The present arrangement of the pipes under the seat gives people hot heads and cold feet.

Crowder estimates there is a sum total of approximately 500 lineal feet of crevices at the edges of doors and windows in a sleeping car. If they averaged one-fiftieth of an inch in width and admitted air at half the rate of the train speed, 40,000 cubic feet of ventilation would be more than accounted for.

Pettenkofer showed that when all visible chinks were closed in a room the rate of ventilation was decreased only 28 per cent. as compared with ordinary closure. A fire burns as well when a room is closed and ventilators shut, some 20,000 cubic feet of air an hour passing up the chimney.

In a dope room fitted with a powerful suction extract fan the closure of six out of eight outlets made no difference to the anemometer readings.

In a dug-out of 900 cubic feet capacity containing 30 soldiers, on a cold winter day, natural ventilation, maintained by the greater temperature and humidity of the air within, sufficed in spite of double gas curtains blocking the exits.

A low room is far better ventilated naturally than a lofty one, and a small than a large one. Height and cubic content are absurd standards to set up in building by-laws.

Experience in temporary army office buildings showed that girls were far more liable to faint in hot weather in rooms 80×70 than in rooms 80×20. The natural wall, door, and window ventilation is much more efficient in the latter.

A factory with light roof construction, very large glass surface, and thin walls cannot be warmed otherwise than by a vast amount of heating surface and fuel in cold weather, while the sun produces the greenhouse effect, to mitigate which fan ventilation is imperative, e.g. fans placed in dormers in the roof, capable of extracting in winter and blowing in air in summer.

In the case of submarines immersed in water experience shows that the vessels can remain submerged until the percentage of carbon dioxide reaches 3. The surrounding water and the metal walls of the

enclosure ensure the temperature not rising to an unendurable height, and the chemical state of the atmosphere determines the period of occupation.

The problem of dug-outs, with walls of varying conducting power and no water jacket constantly renewed, is different, and the wet-bulb temperature may be the determining factor.

Miss Hargood Ash has considered for me the rise of temperature in an enclosed unventilated space occupied by men, and from which no heat is supposed to be lost by radiation or conduction. Let

V = volume of air in cu. metres,
S = specific heat of air,
ρ = density of air,
C = heat in grm. calories produced by one man in one hour,
n = number of men,
N = number of c.c. in vol. V,
t' = initial temperature,
t = final temperature,
H = time in hours,

Then $V \times S \times \rho\,(t-t') = C\,n\,H$.

Taking the heat loss of a man resting or at sedentary occupation, by convection and radiation, as 60 kg. calories per hour and the specific heat of dry air as 0·24 and density 0·0012 at 20° C. and 760 mm. this equation becomes

$$N \times 10^6 \times 0{\cdot}24 \times 0{\cdot}0012 \times (t-t') = 60 \times 1{,}000 \times n\,H$$

$$\text{or} \quad (t-t') = \frac{6}{0{\cdot}24 \times 0{\cdot}12} \frac{n\,H}{N} = 200 \frac{n\,H}{N}.$$

The rise of temperature is then calculated by multiplying the number of men by the number of hours, and by the constant 200, and dividing by the number of cubic metres in the enclosed space.

Example :

Chamber measuring $10 \times 10 \times 2 = 200$ cu. metres.

$$t-t' = \frac{200\,n\,H}{200} = 1°\text{ C. for one man for one hour.}$$

Six men in three hours will raise the temperature 18° C., say from an initial temperature of 10° C. (50° F.) to 28° C. (82·5° F.).

Supposing half the heat produced is lost by ventilation and by conduction through the walls, it would require twelve men to produce the same rise of temperature in the same time ; if three-quarters is lost, fifteen men, and so on in proportion.

The men will evaporate moisture from their bodies and some of this will condense on the walls, giving up the latent heat of evaporation, some of which may be conducted away.

As the temperature rises some of this moisture will again be evaporated, and a portion of the heat given off by the men will be spent on this evaporation. The wet-bulb temperature is of first importance in respect of the comfort of the men in an enclosed space where the air is still.

The wet-bulb temperature must then be considered and how far the six men would saturate the air with moisture in three hours. Let

m' = mass of water in grm. per cu. metre in air at t',
M′ = mass of water in grm. per cu. metre in sat. air at t',
M = mass of water in grm. per cu. metre in sat. air at t,
n = number of men in space,
N = number of c.c. of air in space,
H = time of heat production in hours.

Given one man loses 30 kg. calories of heat per hour by evaporation, or 52 grm. of water,

$$\text{Initial relative humidity at } t' = \frac{m'\text{N}}{\text{M}'\text{N}} \times 100,$$

$$\text{Final relative humidity at } t = \frac{m'\text{N} + 52\,n\text{H}}{\text{MN}} \times 100.$$

Knowing the relative humidity and the temperature of the air, the wet bulb, dewpoint, or absolute humidity can be arrived at with the help of meteorological tables.

Example :

Suppose the walls are dry and the air at the start is 50 per cent. saturated, the dry bulb at 10° C. and the wet bulb at 5·6° C. Taking 580 calories as the latent heat of evaporation and 30 kg. calories as the evaporation loss per man per hour, $\frac{30{,}000}{580} = 52$ grm. of water and $52 \times 6 \times 3 = 936$ grm. water added to the air by 6 men in three hours. At 50 per cent. saturation and 10° C., the air in the space held 933 grm. At the end of three hours, then, supposing the ventilation is nil and no water is condensed on the walls, it holds $933 + 936 = 1{,}869$ grm. If saturated at 28° C. (the temperature the dry bulb is supposed to mount up to) it would hold 5,380 grm. The air is then 35 per cent. saturated, and the wet bulb would stand at 17·8° C. (64° F.).

Six men in three hours would thus increase the dry-bulb temperature from 10 to 28° C., and the wet-bulb from 5·6 to 17·8° C. under the stated conditions.

Supposing the ventilation removed a large portion of the heat produced, and some of the water evaporated, the wet bulb will be raised to the above extent by the space being occupied by a much larger number of men. With wet walls and little ventilation the space will soon become saturated and the wet and dry bulbs rise together.

Taking the carbon dioxide production of a resting man as 14 litres per hour, the six men in three hours would produce 252 litres and raise the concentration of CO_2 to 16 parts in 10,000, an insignificant increase.

In considering loss of heat by conduction through the walls a sphere was taken by Miss Hargood Ash because simpler for mathematical treatment than a cube.

Consider a spherical enclosure with a thick wall, containing a constant source of heat at its centre and the outside surface of the

wall being kept at a constant uniform temperature. Then after a time the spherical shell which forms the wall of the enclosure will attain a steady temperature, and may be considered to be made up of an infinite number of isothermal surfaces which are concentric spherical shells and across each of which the same amount of heat must flow per sec., i.e. the amount of heat produced per second at the centre of the sphere.

Now consider any thin spherical shell in the wall bounded by surfaces of radii r, and $r+dr$ (where dr is a small increment of r). whose temperatures are θ and $\theta+d\theta$ (where $d\theta$ is a small increment of θ), then if the quantity of heat produced per second is Q this amount of heat must flow across the shell considered per second, i.e. across the area $4\pi r^2$; therefore, if K is the conductivity of the material of the wall, we have by definition:

$$Q = -K\ 4\ \pi\ r^2 \frac{d\theta}{dr}$$

$$\text{or} \qquad Q\ \frac{dr}{r^2} = -4\ \pi\ K\ d\theta.$$

Hence

$$Q \int \frac{1}{r^2} dr = -4\ \pi\ K \int d\theta;$$

or if the outside wall be at a distance r_2 from the centre and at a temperature θ_2, r the inside wall at a distance r_1 and temperature θ_1:

$$Q \int_{r_1}^{r_2} \frac{1}{r^2}\ dr = -4\ \pi\ K \int_{\theta_1}^{\theta_2} d\theta$$

$$\text{or} \quad -Q \left[\frac{1}{r}\right]_{r_1}^{r_2} = -4\ \pi\ K\ [\theta]_{\theta_1}^{\theta_2}$$

$$\text{or} \quad Q\left(\frac{1}{r_1} - \frac{1}{r_2}\right) = 4\ \pi\ K\ (\theta_1 - \theta_2)$$

$$\text{or} \quad \theta_1 = \frac{Q}{4\ \pi\ K}\left(\frac{1}{r_1} - \frac{1}{r_2}\right) + \theta_2$$

$$= \frac{Q}{12{\cdot}6\ K}\left(\frac{1}{r_1} - \frac{1}{r_2}\right) + \theta_2.$$

For a volume of 216 cubic metres, i.e. volume given by a cube of side 6 metres, $r = 372$ cm.

If walls are infinitely thick, $\frac{1}{r_2} = 0$.

The value of K is given as 0·004 for the average rock of the earth's crust.

Whether the walls are of metal, stone, sand, or wood, dry or wet, it will make a great difference, as shown by the following:

K = grm. calories conducted per sq. cm. per second across a slab 1 cm. thick, having a temperature gradient of 1° C. per cm. It is for

Sand	0·00013
Cement	0·0007
Wood	0·0004
Slate	0·0047
Marble	0·0071
Average for igneous and sedimentary rocks	0·004
Felt	0·00009
Sawdust	0·00013
Iron	0·150
Water	0·0014

Wet sawdust has about treble the conductivity of dry.

Example 1:

Consider a spherical cavity of 300 cm. radius as a dug-out made in average rock of earth's crust.

Radius of sphere = 300 cm.
Wall infinitely thick (earth's crust).
Conductivity of earth's crust = 0·004.

$$\theta_1 = \frac{Q}{12{\cdot}6} \times \frac{1}{0{\cdot}004} \times \frac{1}{300} + \theta_2$$

$$= \frac{Q}{15{\cdot}1} + \theta_2$$

$$\text{or } \theta_1 - \theta_2 = \frac{Q}{15{\cdot}1}.$$

A man would raise the internal temperature approximately 1° C. in an indefinite period of time, since

$$Q = \frac{60{,}000}{60 \times 60} = 16{\cdot}7 \text{ calories per second.}$$

Example 2:

Consider the same spherical cavity as a dug-out made in sand.

If wall of sand, conductivity = 0·00013:

$$\theta_1 = \frac{Q}{12{\cdot}6} \times \frac{1}{0{\cdot}00013} \times \frac{1}{300} + \theta_2$$

$$= \frac{Q}{0{\cdot}5} + \theta_2$$

$$\text{or } \theta_1 - \theta_2 = \frac{Q}{0{\cdot}5}.$$

A man would raise the internal temperature appproximately 33° C. in an indefinite period of time.

Example 3:

Consider the same spherical cavity of 300 cm. radius with iron wall and surrounded by the sea, which keeps the outer wall at constant temperature, e.g. a submarine. No ventilation is postulated.

Radius of sphere = 300 cm.
Thickness of wall = 3 cm. (steel).
Conductivity of steel = 0·150.

$$\theta_1 = \frac{Q}{12{\cdot}6} \times \frac{1}{0{\cdot}150} \times \frac{3}{300^2} + \theta_2 \text{ very nearly}$$

$$= \frac{Q}{12{\cdot}6 \times 0{\cdot}150 \times 30{,}000} + \theta_2$$

$$= \frac{Q}{56{\cdot}700} + \theta_2$$

$$\text{or } \theta_1 - \theta_2 = \frac{Q}{56{,}700}.$$

A man in this cavity would have no appreciable influence on the internal temperature however long he remained within.

The following are estimates of natural air change in buildings: [1]

For well-constructed brick or stone buildings in sheltered positions with average number of windows and entrance doors	1 to 1½	changes per hour.
Ditto in exposed positions	1½ to 1¾	,,
For frame structures with open roofs of sound construction	2	,,
Ditto with corrugated iron roofs	2½	,,
For building with corrugated iron roofs and sides (according to exposure to prevailing winds)	3 to 4	,,

For the consideration of heat loss through walls, &c., of buildings, Mr. Herring cites the following:

Heat loss coefficients in B.T.U. per sq. foot per degree temperature Fahr. per hour: [2]

Glass single window.	Still air	0·64
,,	Rain, no wind	1·248
,,	Wind, no rain	1·05
,,	Rain and wind	1·485
Double window.	Average	0·4–0·75
Plain brick walls 8 in. thick		0·37–0·46
,, 20 ,,		0·21–0·25
Limestone, sandstone, and concrete walls have a coefficient a little higher than brick.		
Frame, walls, and partitions of ordinary materials and thickness, wood, plaster, &c.		0·21–0·6
Cork boarding 1 in. thick		0·26
,, 3 ,,		0·092
Boards with slag, wool between		0·046
Plaster ceiling / Soft wood floor		0·10
Tile, no boards under		0·902–1·12
Slate with boards		0·30–0·43
Corrugated iron		1·86–2·13
Reinforced concrete floor		0·24

The computation of the total rate of heat loss from a room is an insoluble problem, for there must be considered the transmission losses through walls and the like, and the losses due to air interchange. The dissipation of heat from the outer surface of walls, &c., depends on temperature, wind, and on evaporation if the wall is damp. The escape of warm air depends on crannies, flues, &c. The aspirating effect of a warm building is greatest on the ground floor, least at the top storey; height of building makes a difference both to this and to exposure to wind. Sunshine makes an enormous difference. The conditions, then, are constantly changing.

[1] E. Herring, *Rep. Instit. Heating and Ventil. Engineers*, 1917, **17**, 101.

[2] Multiply by 1·35 to express in calories per sec. per 1 sq. metre of surface.

In order to calculate heat losses in natural air interchanges per hour, the engineer[1] may arbitrarily select a datum room, one with a leakage of one interchange an hour, a top floor room 12 feet high, 18 feet deep, with one long side exposed, having no vent or flue, of normal tight construction, exposure to average winter wind of 20 feet per sec., with 30° F. outside and 60° F. inside, and then add to the one interchange 25 per cent. for basement room (house of three storeys), 30 per cent. for second side exposed, 25 per cent. for depth of room of 12 feet (or take off 20 per cent. for depth of 24 feet), 15 per cent. for each vent or flue. Such computations must obviously be so approximate that skilled hourly supervision of workrooms and control of heating and ventilating is essential.

Observations were carried out by me in a deep dug-out made in sand at Aldershot. The two exits were closed by double gas curtains (wetted) as efficiently as possible. The cubic content was 18·5 cubic metres. The floor was wet. Sixteen persons and two lighted candles were enclosed.

11.35 a.m.	Initial temperatures	Wet bulb 9·5° C.	Dry bulb 10·1° C.
1.15 p.m.	Final temperatures	Wet bulb 15·2	Dry bulb 15·9

The carbonic acid rose at the end of the period to 1·5 per cent. The day was a cool, breezy, and rainy one. The natural ventilation sufficed to keep the dug-out comfortable with an air volume of about 1 cubic metre per person. If the observation had continued the CO_2 concentration might perhaps have risen to an uncomfortable height.

Further observations were made in a deep dug-out in chalk at Chatham. There were three exits all closed with double gas curtains, and crevices were stopped with sandbags and clay. The closure was considered by the officer in control (a mining expert) to be better than is usual at the Front. There were, however, crevices which allowed a perceptible draught. The cubic content was approximately 26 cubic metres; 30 persons and three lighted candles were enclosed.

11.40 a.m.	Initial temperatures	Wet bulb 9·8° C.	Dry bulb 10·5° C.
1.20 p.m.	Final temperatures	Wet bulb 13·4	Dry bulb 17·4

The carbonic acid rose to 0·6 per cent. by noon and remained in equilibrium. The heat conduction through the walls, together with the natural ventilation on a clear cold day with a good breeze blowing, which was set up by the difference of temperature, sufficed to keep this dug-out in a comfortable condition when crowded with 30 men, with approximately an air volume of 1 cubic metre per man.

The efficiency of such natural ventilation depends mainly on difference of temperature inside and out (humidity is a factor), and therefore may be almost nil in hot weather.[1]

Robertson, discussing the ventilation of cinema theatres, says a cylindrical shaft 1 foot in diameter, with a velocity which rarely exceeds 200 feet per minute,[2] may carry off 9,600 cubic feet per hour. To change the air only once an hour in a building of about 120,000 cubic feet capacity, accommodating 1,000 people, twelve such shafts are required. Such shafts are totally inadequate in still, warm weather.

[1] Ventilation by diffusion through a number of small openings is very small in the case of a chamber placed in a room, the temperature being the same.]

[2] W. Nobbs, *Instit. of Heating and Ventil. Engineers*, June 24, 1919.

A Conversion Table of Velocities.

Metres per sec.	Miles per hour.	Feet per sec.	Feet per min.
10·0	23·37	32·80	1,968
4·47	10·0	14·6	880
3·048	6·818	10·0	600
0·508	1·136	1·6	100

For estimating the time required for the clearance, by means of an extract fan, of a closed room or dug-out of a poison gas, the following calculation has been given :

Let the amount of gas present in the chamber at any time t be a, and that present at a later time $(t+\delta t)$ be $(a+\delta a)$. Let the volume of the chamber be v litres and the capacity of the extract fan be c litres per minute. Then in time t the volume of gas extracted by the fan will be $\frac{a}{v} c\, t$ litres, and in a time $(t+\delta t)$ the volume will be $\frac{a}{v} c\,(t+\delta t)$; but the amount extracted during this time will be $a-(a+\delta a)$, therefore equating

$$a-(a+\delta a) = \frac{a}{v} c\,(t+\delta t) - \frac{a}{v} c\, t$$

or $$-\delta a = \frac{a}{v} c\, \delta t\,;$$

$\therefore$ when $\delta t \longrightarrow 0$

$$\frac{da}{dt} = -\frac{a}{v} c$$

and $$\int \frac{1}{a} da = -\frac{c}{v} \int dt.$$

Integrating both sides of the equation :

$$\log_e a = -\frac{c}{v} t + \mathrm{K},$$

where K = constant of integration,

or $$a = e^{-\frac{c}{v} t + \mathrm{K}} = e^{-\frac{c}{v} t} \times e^{\mathrm{K}}$$

$$= \mathrm{K}_1\, e^{-\frac{c}{v} t}, \quad \text{where } \mathrm{K}_1 = e^{\mathrm{K}},$$

when $t = 0_1$, $a = a_0$;

$\therefore$ $\mathrm{K}_1 = a_0$

and we get

$$a_t = a_0\, e^{-\frac{ct}{v}}$$

or $$\frac{a_0}{a_t} = e^{\frac{ct}{v}}.$$

To find the time in which the concentration of the gas is reduced to one-half of its original value, the above equation may be written

$$\log_e \frac{a_0}{a_t} = \frac{ct}{v}$$

or $$\log_e \frac{1}{\frac{1}{2}} = \frac{ct}{v}\,;$$

hence $$t = \frac{v}{c} \log_e 2.$$

For aeroplane doping rooms the Home Office requirements are that the air must be changed three times an hour by fans (preferably extract) with outlets at floor level, and the total area of inlets no less than three times the area of outlets.

The New York Commission on ventilation bases the changes of air required in schools on the heat production of the pupils. One of the members of the Commission, D. D. Kimball, calculates the heat given off by the pupils as 300 B.Th.U.[1] per pupil per hour, sitting in the standard American class-room. Each pupil gives off enough to heat 1,800 cubic feet of air per hour through 10° F. He says this volume of air per pupil properly introduced and diffused throughout the occupied space of the room will absorb and carry off the heat and moisture given off by the pupils and give satisfactory ventilation.

When a plenum system is used there is an additional natural ventilation through crevices, and heat is lost to the walls. 1,800 cubic feet per hour is, however, not enough when artificial lights are lit, or when the sun shines upon a room and the air is warm in summer, nor will it be enough in winter in factories wherein machinery and furnaces develop heat.

This is shown by the following figures given by Grierson :[2]

Heat production of a man	350	B.Th.U. per hour.
Each horse-power used in overcoming friction in machinery	2,530	,,
Each Board of Trade unit of electricity expended in friction	3,400	,,
An incandescent gas burner consuming $3\frac{1}{2}$ cu. ft. of gas per hour	1,925	,,

S. Rideal[3] gives the heat production of a 25 c.p. gas lamp as 691 B.Th.U. per hour, the moisture production in grains as 506, the CO_2 production as 0·77 cubic feet. The heat produced by a 25 c.p. electric lamp he gives as 329 B.Th.U.

Grierson takes one B.Th.U. as raising the temperature of about 53 cubic feet of air at 47·5° F. through 1° F.

Supposing the heat production per man with his share of gaslight and his machine were 1,500 B.Th.U. per hour, and allowing the temperature of the air to rise 5° F., the air requirement would be $\frac{53 \times 1{,}500}{5} = 15{,}900$ cubic feet of air per hour per man when neglecting wholly any heat loss through walls, &c. Taking this into account and supposing the walls and roof carried away half the heat produced, it is clear that a very large volume of air is required to keep down the temperature.

Unclouded sun shining on a 13-inch brick wall transmits, according to Grierson, 6 B.Th.U. per sq. foot per hour, and a glass surface 150 B.Th.U. From a room with a sun-lit glass area 100×100 ft. = 10,000 sq. ft., 1,500,000 B.Th.U. must be removed per hour to prevent rise of temperature.

One-storey factories are, then, built with skylights facing north, and should have a double roof with ventilated air space between facing south. In workrooms and factories exposed to the sun there

[1] 3·97 B.Th.U. = 1 Cal.

[2] *Modern Methods of Ventilation*, Constable, 1916.

[3] *Proc. Nat. Gas Congress*, Oct. 14, 1913.

should be provided outside sun-blinds, and fans to impel air over the workers.

The following are the chief systems of heating and ventilation:

1. Open fire, coal or gas, and ventilation by flue, crannies, and window. Tobin tubes may be added.

2. Hot-water pipes, or steam coils, or anthracite stove, and ventilation by crannies and window. Sometimes ventilator openings are made at the back of and under hot-water coils. Fans may be used in addition to stir the air or to extract.

3. Gravity system. Air admitted at the basement and heated there rises up flues into rooms. Vent flues may be added and heaters placed in these to accelerate the air current.

4. Plenum system with single fan impelling heated air up flues into rooms.

5. Double-fan plenum, one impelling warm air, the other extracting through flues. Direct heating by water coils may be added.

Winslow says, when a room is so crowded that the floor area is less than 200 sq. ft. per head, either a fan exhaust of 30 cu. ft. per capita or windows open to the extent of 0·25 sq. ft. per head will usually be necessary to secure a reasonable air change. This is shown by the evidence of the following table:

TABLE XXVIII.—*Relation of Artificial and Natural Ventilation to Air Conditions, New York Office Building. Number of Rooms of each type showing a given Carbon Dioxide Content* (C. E. A. Winslow).

Carbon dioxide parts per 10,000 .	4	5	6	7	8	9	10	11	12	13	14	15	16
Rooms with exhaust over 30 cu. ft., window openings over 0·25 sq. ft. p.c.	5	9	8	6	2								
Rooms with exhaust over 30 cu. ft., window openings under 0·25 sq.ft. p.c.		1	1	2	1	1							
Rooms with exhaust under 30 cu. ft., window openings over 0·25 sq. ft. p.c.			12	14	11	2	2		1	1			
Rooms with exhaust under 30 cu. ft., window openings under 0·25 sq. ft. p.c.	1	1	3	6	6	4	13	11	5	6	2		1

There is no doubt that the radiant heat of an open fire and the ventilation maintained by it, and by an open window when necessary, is the most healthful in this climate of mist and cloud. The radiant energy of the fire makes up for the absence of sunlight.

The great advantage derived from radiant heat and wide openings in the roofs is shown in some of the best-planned modern foundries, forge and annealing shops, wherein the cooling conditions are excellent, a draught being maintained by the cool, outside air sinking in and displacing the air heated by the furnaces.

In a foundry not ventilated by such roof openings the dry bulb was 92°–95°, the wet bulb 72°–74° F., the dry kata and the wet kata cooling power varied from 25 to 11 near where a caster was pouring a crucible.

Ventilation of one-storey serrated roofed factories by the opening here and there of skylights is a very imperfect method. Draughts are felt and the openings shut, or it is nobody's business to open them.

Narrow concealed openings running continuously along each of the troughs of the serrated roof are far more efficacious. These are always open, and add greatly to the efficiency of workers. In cold weather extra heat in the steam pipes must suffice to compensate for the entry of the cold air.

Fans placed in dormers in the roof, made to extract in winter and blow in during warm weather, are a very effective addition to such factories. Machines and furnaces which are sources of heat can in many cases be enclosed in light screens and ventilated so that the heat does not enter the workshop.

One often finds, on inspecting factories, men at work in hot shops who have nothing to do with hot processes, and should, therefore, be in cool factories.

In machine shops, decomposition of oil and soap-suds used for machines leads to a close smell, which workers imbued with the popular and erroneous chemical theory of ventilation regard as poisonous. It is the stagnant, monotonous, and often over-warm atmosphere they suffer from.

In browning and hardening shops badly ventilated I have found wet kata cooling powers of 12–13, and dry kata powers nil.

A young man may be engaged under a hood in front of a furnace plunging component parts in a bath of oil and shaking them in a blazing sieve before plunging them into water. For eight hours a day he may spend his youth and vigour in such excessively warm and dirty work. It seems desirable that he should not only have a cold spray of air provided but that alternation of work should be arranged, to prevent fatigue and secure greater enjoyment of life and output.

It is common knowledge that one may remain in a room and not be aware of the increasing rise of temperature. In the case of schools, if control is left to the teacher, listlessness, inattention, and ill-health result in the pupils. The beneficent effect of the open-air school is illustrated by the following, taken from the Report of the Chief Medical Officer of the Board of Education.

'Most of the children are, on entering the school, dull, listless, anaemic, and dejected. After a few weeks at the open-air school they are bright and alert, quick to perceive a joke, and full of the joy of life that is their birthright. Pure fresh air, rest, cleanliness, and wholesome food have performed their usual miracle.

'Children specially selected by reason of their habitual delicacy not only survive the ordeal of the open-air school through a winter of such exceptional severity as 1916–17, but manifest a most astonishing access of health and vigour.'

'The majority of children,' writes a head master, 'beginning at the age of five, spend nine years in the public elementary schools. Hundreds of these schools, especially in the country and the smaller towns, want blowing up. The councils will not spend a penny on buildings not handed over to them. The voluntary schools are in the hands of people who cling to them tenaciously, and cannot, or will not, put them into proper condition. Many are insufficiently heated, and to counteract this windows and doors are kept closed, and the class-rooms are mild "black holes".'

Thirty years ago Drysdale introduced air into three schools by fans

worked by a gas engine through vertical shafts, these opening by registers into the upper parts of the schoolrooms. Holes were made in the floor at frequent intervals, and these were connected with the outside air by openings in the walls. As a consequence, sleepiness and inattention was almost entirely removed and the health of the children improved, and the school gained the highest Government grant in the district.

D. McKail [1] says that for schools in the British Isles where the air is tempered and propelled by fans into rooms, the temperature of the air should not exceed 58° F. dry and 51° F. wet bulb. The success of the system depends on the careful control of the air temperature, a difficult matter in our changeable climate.

In contrast, the Chicago schools are kept at 72° F.; in older installations the temperature is often 75° F., and because 1,800 cubic feet of air is supplied per pupil per hour, it is assumed ventilation is as it should be—an assumption rarely justified according to Dr. Evans of that city.

Winslow and Baskerville carried out 1,854 observations of temperature and humidity in New York schools, and found 14 per cent. over 71° F., 5 per cent. over 73° F. Of 773 carbon dioxide determinations, 15 per cent. were over 10·5 and 6 per cent. over 12·5 parts per 10,000.

The average results for all fan-ventilated schools were about the same for all window-ventilated schools. The window-ventilated were all mediocre; some of the fan-ventilated were admirable, and some very poor—mainly from careless operation.

In a well-managed fan-ventilated school 3 per cent. of the readings were over 71° F.

Dr. E. E. Smith writes to me from New York (Department of Education):

'There can be little question that the habit of heating houses in America to so high a temperature has developed from the extremes of weather conditions. Because we occasionally need a considerable heat to offset a low outside temperature we habitually tolerate an undesirably high inside temperature. Our women dress accordingly, supplying an excuse for the continuance of the habit. Indeed, our offices and homes are more frequently heated above 70° than below. If heat were not supplied to bring the temperature above 60° in a modern office or home in this city, it would, I believe, be regarded by the courts as sufficient grounds for breaking the lease of such property.

'So far as my personal observation goes, 70° is holding its own as the indoor temperature desired by the American people. That they do need more heat than the English people is quite probable from our climatic conditions. That because of this they have developed the habit of too high a temperature, particularly in the homes, I believe we must admit.'

The cold air of an American winter, when heated to 70° F. and reduced in relative humidity to some 25 per cent., by increasing evaporation makes people feel colder than air at 64° F. of average humidity (Huntington).

Owing to the high summer temperature the citizens of U.S.A. become set to a tropical climate, and this also makes them demand

[1] *Public Health*, June 1900.

higher temperature in winter. On coming to England they have to put on fat to secure comfort.

In the case of schools, offices, and factories there is difficulty in maintaining air supply through open windows on days when there is no breeze, and on the leeward sides of buildings, and of maintaining it without undue draught when there is breeze.

Schools built on the hospital block plan, with hopper windows on either side, secure efficient cross-circulation, but much heating surface and large expenditure of fuel is required when radiators are placed round the walls.

Much can be done by throwing open schools and factories in intervals of work. The effect of throwing open all doors in an interval is shown in the case of a cinema theatre where the carbon dioxide sank from 44 to 17½ parts per 10,000.

Gauze screens can be used to break down draughts from open windows and skylights. If hot-water pipes are placed on or under the floor so as to warm the feet in the manner of the Roman hypocaust, cool air from open windows can be borne with comfort, or a definite rate of movement established by extract fans and suitably placed inlets.

The fans being reversible so that air can be blown in, and all windows opened in warm weather, most suitable conditions may be obtained for workers.

As the movement of the air, not the number of changes of air per hour, is the essential factor, the direction of mechanical ventilation makes all the difference. Thus by proper direction of current from inlets and volume delivery of extract-fans rooms can be mechanically ventilated without the expense of a plenum system.

Suppose, for example, a workroom is in feet $100 \times 50 \times 10 = 50{,}000$ cu. ft. capacity.

To obtain five changes of air per hour by fan ventilation $\frac{50{,}000 \times 5}{60}$ = 4,166 cu. ft. per min. is required.

The cross-sectional area of the room, supposing the current goes lengthwise, is $50 \times 10 = 500$ sq. ft.; the mean velocity of current is then $\frac{4{,}166}{500} = 8{\cdot}03$ ft. per min.

But supposing the current were arranged to go crosswise, then the sectional area is $100 \times 10 = 1{,}000$ sq. ft., and the mean velocity is $\frac{4{,}166}{1{,}000} = 4{\cdot}6$ ft. per min.

Supposing the temperature of the room were kept at 60° F. and a dry kata cooling power were required of 7, five changes per hour would give too high a cooling power if the current traversed the room lengthwise from inlet to exit, but the right cooling power if it traversed it crosswise.

The effect of a fan put to stir the air in a factory is shown by the following:

Dry kata	Wet kata	Dry bulb	
4·0	15·0	23·5° C.	Fan off
6·0	25·5	23·5	Fan on

Unpleasant draught from such a fan can be prevented by using revolving or reversible fans which blow first this way and then that.

By means of blowers and ducts a cold spray of air can be supplied to and put under control of the individual worker. These could be used with great advantage in places where the workers have to face great heat, e.g. tin-plate rolling shops, glass-blowing shops, manoeuvring platforms of torpedo ships, furnace- and engine-rooms.

The ventilation of warm places, such as furnace-rooms, engine-rooms in ships, can be effected by fans placed at the bottom of wide vertical air trunks (fan and trunk some 6 ft. in diameter), down which the cool outside air naturally sinks, the fan construction being such as to

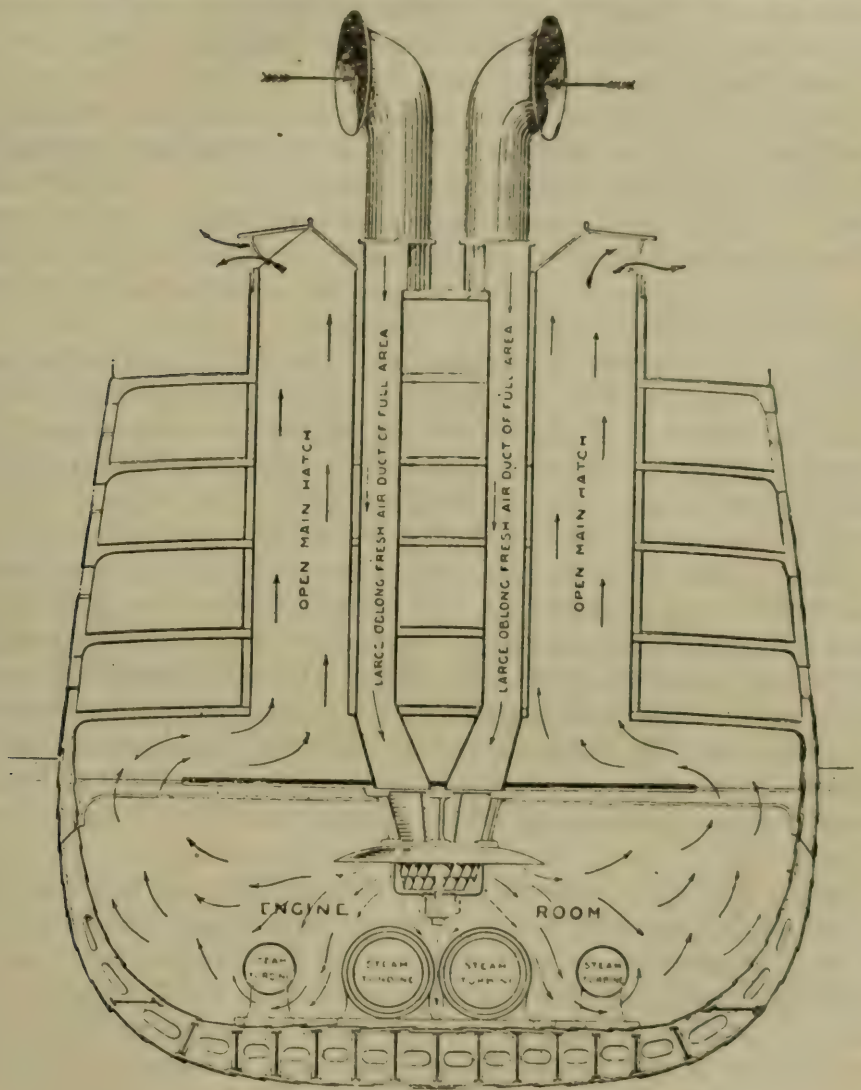


Fig. 47.

break the air up into such fine streams and impel it in all directions so that no draught is felt. In the case of the Cunard liners, Mr. Keith tells me, a temperature of 150° F. when the engine-room was in full swing has been reduced to 70° F., and the whole cubical air contents of the central turbine-room changed to the extent of 80 times an hour. 'We are actually giving this room 6 million cu. ft. more fresh air per hour, and that too without appreciable draught.' The fans are called 'joy wheels', Fig. 47.

In the case of rooms ventilated by open windows and heated by radiators it is no one's business to see that proper conditions are

secured. In the case of schools, factories, &c., there should be a skilled attendant, who should give his whole attention to this. Owing to the want of such skilled attention, ventilating engineers claim that a well-managed balanced plenum system gives the better results.

A. E. Tebb[1] reporting of factories of class A in Birmingham, says the apportionment of windows is very liberal, and all measures and apparatus for their proper opening and adjustment are usually in good order, so that in the great majority of instances effective 'natural ventilation' by this means should be obtainable under all ordinary circumstances. Unfortunately, however, and this applies also very largely to heating, while installations are provided—often at great cost—their regulation is fortuitous.

In one shop an old bronchitic male, in another some weak anaemic girl, both with an equal horror of fresh invigorating air, dominate the ventilation and heating arrangements for a hundred or more healthy persons. It is no uncommon thing to see hundreds of girls trooping out of workrooms, into the cold night air, with flushed and perspiring faces. It is no uncommon thing to pass factory after factory without seeing a single window open.

The notorious failure of mechanical systems of ventilation in the past is attributed to lack of adequate expenditure or knowledge on the part of engineers in the providing of an adequate plant, and in the absence of proper operation, arising from ignorant janitors, or false economy of fuel on the part of officials in public buildings.

In the case of an office building Winslow found 31 per cent. of the register areas inadequate; 52 per cent. of 213 thermostats were not controlling their radiators. In certain ducts designed for fan ventilation no fans were installed, while in others fans were running at only a fraction of their efficient speed. In other ducts the register openings were so badly adjusted to the present population, that while some rooms received more air than they needed, others received much less. In a plenum system where the inlets were placed too high I found the dry kata reading was 5 at head level and 8 four ft. above head level.

In many hospitals, says Winslow, the operation of the ventilating plant is left in unskilled hands, with no supervision until bad conditions force themselves on the senses, and then the whole system is discarded without an attempt to find out what is really wrong.

In one hospital in New York, notorious for its over-heating, three records of 70–74, and two of 75 and over were obtained. Five out of eleven records in children's wards were over 70° F.

In a hospital in Chicago, greatly troubled by odours in the wards, the fresh-air intake on the roof opened just over the soil pipe of the plumbing system, and drew its foul air directly into the ventilating ducts.

In some factories and some of the great Atlantic liners air is propelled by fans placed at the intake on deck into the rooms through ducts which are long, tortuous, and narrow. The volume of air

[1] M. Greenwood and A. E. Tebb, 'An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to female Munition Workers', M. R. C. *Special Report Series*, No. 22, 1919.

delivered, owing to friction, is far too small, and, no exhaust fans being supplied, the ventilation is most deficient in spite of a most costly equipment. In British warships air is extracted by trunks and fans, and fresh air finds its way in—a far more efficient ventilation without draught is thus secured.

In the House of Commons 20,000 to 40,000 cu. ft. of air per min. are forced through the perforated floor of the chamber. Dust from the boots of members is carried up by the air-current from the floor, but there is no evidence that such dust is worse than in rooms where there is no plenum system. Catarrhal infection from one member to another while in the chamber is greatly lessened by the up-current. The velocity of the incoming air, heated to 63° F., cools the feet and legs of the members by sweeping over or through their footgear and trousers. The body responds by shutting down the blood-supply to the skin in these parts, which feel cold, while the mucous membrane of the nose becomes congested and swollen, and in consequence the head feels stuffy, particularly in those subject to nasal catarrh.

Kata-thermometer readings showed that the cooling power was 50 per cent. greater at the foot than at the head level.

The Ventilation Committee, sitting just before the war, allowed me to make an experimental trial of introducing the air at the gallery level, and shutting up the floor inlets in a section of the chamber. When the full velocity was used the air-current swept from the gallery right across the House, and could be felt on the face when sitting on the back benches of the opposite side. By reducing the velocity and varying the temperature of this air, the conditions could be made pleasant and comfortable with a scarcely perceptible movement round the head. Sitting where the old system of ventilation was in action my feet were chilled, and left nasal airway (narrowed by a deflected septum) was blocked up in two or three minutes. In the part ventilated by the new system the nasal airway became free, my feet warm, and head cool. The war and need for economy has prevented the trial of this new installation on the large scale.

As the heating and ventilating system is the basis of health, comfort, and efficiency of the workers, a building should be designed to secure efficiency of this in the first place, architectural ornament taking the subsidiary position. The ventilating engineers say that the common practice is for the artist to plan the building, including the decorative features, and leave the elevator, water, gas, sewerage, ventilating, heating, and telephone engineers to do their best (or worst), fighting their every request for space.

On the other hand, the common-sense plan is followed by N. S. Thompson (of the Supervising Architects' Office, Treasury Department, New York) of instructing the architectural draughtsmen that it is their duty to arrange the building to receive the proper mechanical equipment as indicated by the mechanical draughtsmen on the preliminary drawings, showing the arrangements of the building.

Unless very great care is exercised in the design and construction of the ducts and registers, the mechanical ventilating system is doomed to failure. Any increase in the length of duct increases the frictional loss, any abrupt change in sectional area or direction converts kinetic

energy of flow into heat by reason of eddies and whirls. 'The duct work cannot be tucked into any nook or corner, or altered in area or direction to suit any and every tradesman engaged on the job. The air distribution system is part of a definite and highly intricate machine, and must be treated with as much respect as a dynamo, or big printing machine.' (Grierson.)

In the mechanical system advocated by the consensus of opinion of the leading ventilating engineers, the rooms are heated by direct 'radiators', and ventilated by air tempered and conditioned, which is both impelled and extracted by fans.

The good results obtained by such a system are illustrated at the Cambridge large examination hall, 106 feet by 51 feet, seating 1,000. Radiators heated by hot water are placed therein near the walls at floor level. The hot surface must be kept at as low a temperature as possible, and be widely distributed near the floor. Fresh air is impelled by a fan through two rows of gratings placed all along the two longer sides of the hall at the height of 8–9 feet above floor level. In the ceiling along the central line are seven large openings through which air is drawn by an outlet fan.

Experience shows that the cooler the air sent in the pleasanter the atmosphere, and that when the hall is full it is difficult to send in air cool enough even when the outside temperature is as low as 40° F. The oppression felt in rooms warmed by hot air is altogether absent. In contrast, in one of the Cambridge laboratories, warmed by fresh air passed over steam-heated pipes, the oppression and discomfort felt is such that some of the professors stop up the ducts.[1]

Supposing that to maintain the temperature of a room at 60° F. the introduction of 15,000 cu. ft. per min. of air heated to 120° F. is required. This air yielding its heat to the surface of the occupants, in particular to the naked skin of the face, will give uncomfortable feelings, congest the nose, depress the metabolism. Cold surfaces and hot air produce a well of cold air at floor level. Hence the discomfort of hot-air systems of heating.

Air introduced at a temperature more than 10° F. less than room temperature is apt to occasion annoying draughts unless very widely and equally diffused, hence the importance of wide and widely distributed registers, as in the Cambridge examination hall.

For cinema theatres J. Robertson recommended that efficient extraction fans should be kept continuously at work during the performance; that adequate inlet openings should be provided and kept open in such situations as would permit of uniform distribution of the fresh air; that during cold weather this incoming air should be heated, otherwise unbearable draughts would be experienced. This last statement is not borne out by the Cambridge experience.

By the balanced plenum system recommended by ventilating engineers, cold air is drawn in from an area remote from sources of contamination through a protected opening and along a glazed brick shaft to the basement, where it is warmed by a coil to the required degree for humidification, and then passed through a washer where it is cleaned and humified (and cooled in summer), and so to the reheat-

[1] J. B. Lock in a letter to *The Times*.

ing coils for final heating. The air is then drawn into the fan and sent along the ducts and through the register into the apartments. A second fan extracts through registers in the ceiling. A slightly larger volume of air is sent in than is extracted.

D. D. Kemball (New York Commission on Ventilation) says the installation of a number of small fan units is much preferable to the use of a less number of large fans; a small system is easier understood by the janitor, and a number of such rather than a single large plant is more flexible and satisfactory. Automatic temperature control should be regarded as a necessity, resulting not only in 10–20 per cent. fuel saving, but preventing excessive temperatures in rooms. Direct radiators and mixing dampers must both be regulated by the thermostat so that they do not act against each other.

We may remind the reader here of the number of thermostats found out of order by Winslow in the New York store. Such a system without proper upkeep is far worse than useless. Kemball recommends the ventilation of a school (U.S.A.) to be carried out as follows: The plenum chamber is divided into upper and lower parts, the temperature of the air passing through the upper chamber being slightly higher than that required for ventilation of the rooms, and the temperature of the air in the lower chamber being approximately 20° F. less. Connexions are made from both of these chambers to the individual ducts with mixing dampers in each connexion automatically controlled by a thermostat located in the schoolroom. In addition, volume dampers are installed in each duct to regulate the volume of air supplied to each room. The mixing and volume dampers are placed at the plenum chamber, assuring better attention on the part of the janitor. Exactly the required volume of air at the exact temperature demanded by any and every individual room may be had as required, e.g. to suit those warmed by the sun and those in the shade and subjected to a cold wind.

Big halls require mechanical ventilation more than small rooms because the mass of air in them is widely separated from the crevices of doors and windows and the porous wall surface. It must be borne in mind that volume increases as the cube, surface as the square.

If the volumes of a cube are . .	1	8	27
the surfaces are	6	24	54
the ratio of increase of surface .	1	4	9

Kemball, discussing church ventilation, says no occupant in an auditorium should be farther than 25 ft. from a fresh-air register, or nearer than 6 ft. to a vent register, and there should not be less than one fresh-air and one vent opening to each thirty occupants. In so far as the number of fresh-air and vent openings are increased and generally distributed the satisfaction will be increased. In warm weather all windows are opened and all the fans made to blow into the auditorium.

In large rooms such as auditoria the air should be exhausted through mushroom openings at the floor and introduced through ceiling and wall openings. This is to avoid cooling people by the incoming air, which has to be 10°–15° cooler than the room temperature.

I am informed that in American hotels provided with a modern

plenum system, by turning a handle one can set the temperature of one's room at 60° or 70°. The windows of these hotels are sealed, and the air, screened through water sprays, is introduced free from odour and 'conditioned' in temperature and humidity. By such means the block of offices adjoining the stock yards in Chicago are rendered free from the smell of the slaughter houses which pervades the outside air.[1]

The New York Commission on Ventilation used small tissue paper flags suspended on a silk thread to detect movement of the air in schoolrooms. Air currents even as slight as 5 ft. per minute cause a deflection, and the extent of this in inches away from the perpendicular can be read.

The survey is carried out by means of a travelling crane at ceiling-level, from which a cross-bar is suspended to which the flags are hung. By means of ropes and pulleys the room is explored at 3 inches from the floor, 3 ft., 6 ft., 9 ft., and 11 ft., the room being 14 ft. in height. A total of 1,030 readings is made for the five planes into which the schoolroom is marked out. Fig. 48 shows the kind of results obtained. The white area indicates no deflection, the lightly shaded deflection up to 3 inches from the vertical, the medium shaded 4 to 6 inches, the darkly shaded 7 inches and over.

In a schoolroom with individual inlets beneath each desk, or nearly 50 in all, and six outlets spread over the ceiling, the circulation of air is so equable and at such slight velocity as barely to deflect the flags.

The plenum air is usually introduced through wall registers at a height of 8 ft. above floor-level. As already stated, these registers must be wide and placed all round the walls.

Kemball says the air washer increases efficiency of the ventilating plant by at least 25 per cent. It has certainly an important function in removing dust and in cooling the air in summer. In the dry frosty air of North America humification allows the air to be less heated, because the more humid air cools the body less by evaporation.

The mean temperature in Chicago in January is 8° F. below freezing, and in July 72·4° F. In Dakota the thermometer has registered 106° F. and −44° F. On the other hand, in the British Isles a mild, moist climate pertains, occasionally and suddenly varied by cold.

Hot-water—or steam—coil valves are turned full on on a cold frosty day, and left full on when the frost turns to a mild, still, humid weather next day. It is nobody's business to see to this, and the results are most enervating.

Expensive plenum systems are not necessary in this country if proper control of heating coils, windows, and extract fans is kept up by a skilled person appointed to attend to the matter. Plenum systems are of value in so far as they effectively replace lack of control. The best plenum system fails in warm weather if the fans cannot be made to blow in and all windows opened.

[1] The cost of the best kind of plenum plant before the war in America is given by Kemball as averaging 3·1 cents per cu. ft. of building.

In a factory where there was a plenum system the dry kata cooling power was 3 to 4 in warm weather, and the operatives were impelled by the close conditions to break the windows.

In a part of the same factory ventilated by windows the dry kata cooling power was 7 to 9.

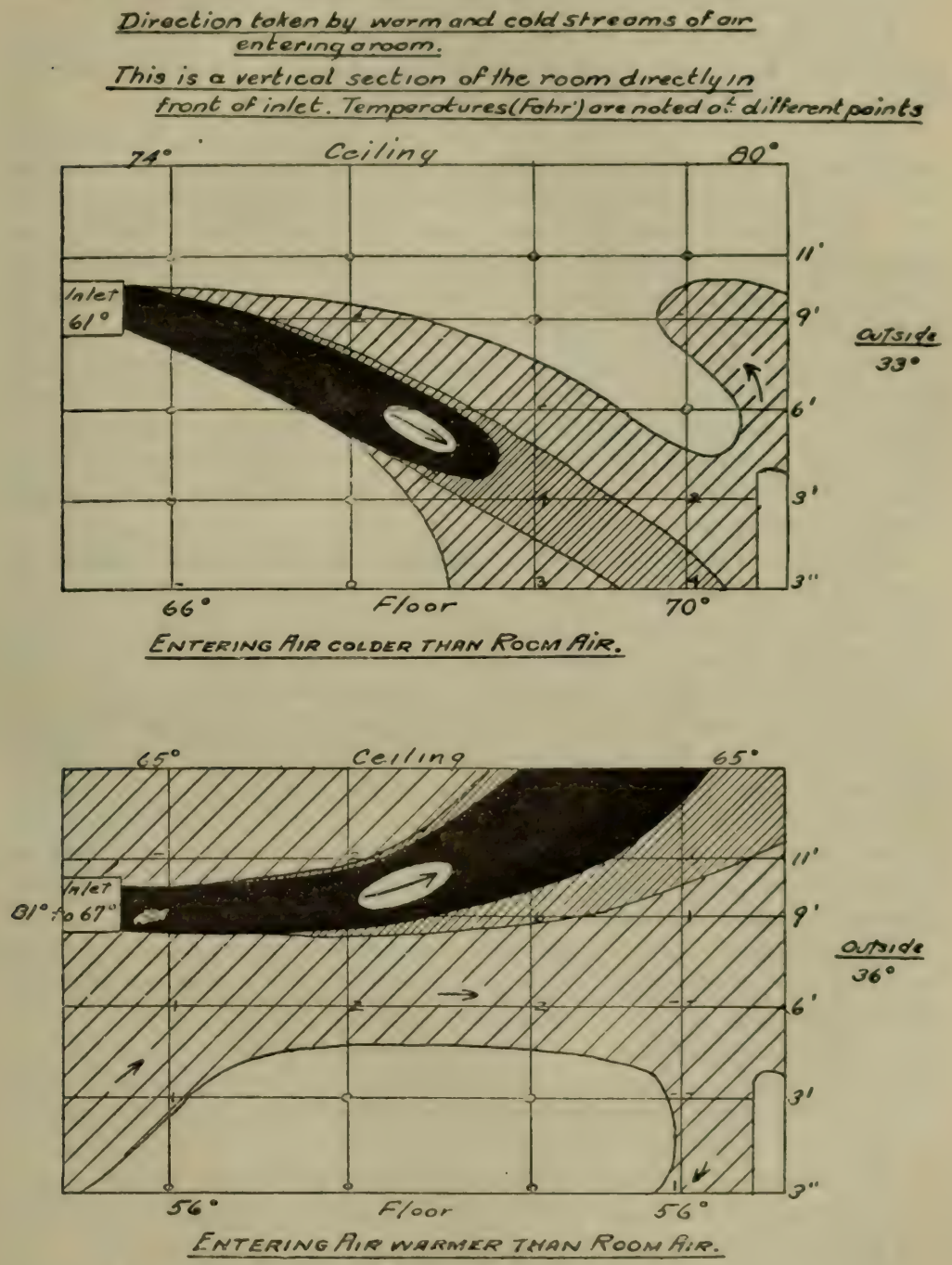


FIG. 48.

Recirculation of air has been used with success in America, where the excessive cold of the winter air makes the conditions quite different from those in the British Isles.

The air is passed through a chamber in which water is sprayed by forcing it out of perforated copper pipes or through nozzles; a curtain may be formed by the sprays intersecting. The water is circulated by

a pump and may be used over and over again for some time. Such washers removed from 37 to 88 per cent. of bacteria, and 27 to 87 per cent. of dust particles.

The recirculation saved 40 per cent. of the cost of heating when the outside air was 32° F. The trifling increase of carbon dioxide was of no account, and the washed air was sent back cool and sweet.

For cooling tropical houses compressed-air douches could be used, as air escaping from compression is both cool and dry. Fans and punkahs are the ready means of cooling in hot weather.

In the tropics the house must be set open by night to cool, and closed up in the heat of the day, the air within being then blown about by fans. By this means a house has been kept with the dry bulb 30° C. lower than the outside air. Tropical houses should have thick walls (mud bricks are excellent) painted white, with double roofs (reed thatching is excellent), the space between the roofs ventilated (ventilators protected by copper gauze against insects). The effect of a coat of limewash containing 15 per cent. of vegetable oil was to lower the temperature under a roof from 124 to 98° F. Wide verandahs, double roofed and ventilated, should protect the sun-exposed side. The house should be orientated so that the prevailing wind blows through it, ample inlets and exits being provided in each room, and louvred shutters to these.

The greater portion of the dwellings in North Queensland for example are unsuitable, built as they are of galvanized iron on a wooden framework, without proper insulation from the sun.[1] Towns are not laid out so as to secure the maximal windage. Women thus work in an atmosphere far warmer than that out of doors.

It is not generally recognized that the successful working of drying-rooms in laundries and factories depends wholly on their being supplied with an ample through current of dry air, not on heat as is often supposed. While natural ventilation is kept up by the heat of the drying-room, exact control can be obtained by heating the material to be dried and ventilating with air of known humidity and velocity by means of a fan.

It has already been stated that ozone does not exist in the lower region of the atmosphere, but traces of oxides of nitrogen are produced therein by the action of sunlight. Ozone is an irritant to the respiratory membrane in a concentration of one part per million of air. It can be safely used in ventilation for destroying odours in concentrations just perceptible to the smell, i.e. less than one part in ten millions of air.

For the removal or masking of offensive smells ozone has a use, e.g. in the chimney-stacks of fish glue and fertilizer factories, in urinals and tube railways.

The slightly irritating effect of ozone may help to keep off catarrhal infections in those who breathe ozonized air. While such air has no power to destroy bacteria it may delay the growth of such, and of moulds in the surface layer of meat in cold storage.

A. H. Barker has set forth the following as a rough practical basis of comparison of cost between different systems of heating giving the

[1] Whitewashing or painting white the outer surface makes a great difference.

same feeling of warmth and maintaining the same interchange of air approximately :

Approximate equivalents	Relative cost
100 B.O.T. units of electrical energy in best radiant stove	100–400
1·38 tons of best house coal burned in bad grate	29–39
0·52 tons of best coal in best grate	10·8–14·6
0·235 tons of best anthracite burned in best slow-combustion stove	7·54–9·42
18,300 cu. ft. of gas burned in medium old-fashioned gas fire	36·6–44·0
10,700 cu. ft. of gas burned in best gas fire	21·3–25·6
0·198 tons of coke burned in best water boiler with well-clothed circulation to radiators or pipes	3·17–4·75
0·306 tons of coke burned in usual small house hot-water installation	4·9–7·36
19·5 gallons of petroleum burned in stove discharging combustion products into room	10·4–13·0

In the case of an electric stove, or of an oil stove or gas radiator delivering products of combustion into the room, or electric, gas, or oil lamps, the heating efficiency of the electricity consumed or fuel burnt is nearly 100 per cent. It must be remembered that only about 8 per cent. of the energy value of the coal used to produce it is obtained as electric energy. The moist, stagnant, warm atmosphere of such a room, from the point of view of health and stimulus to working, has a low efficiency.

By central heating installations it is claimed 50 to 70 per cent. of the thermal energy of the fuel goes to warm the rooms. To conserve heat it is recommended [1] that double windows, weathering strips, draught excluders be fitted, and windows not opened, in other words the recommendations are heated air and a minimum of ventilation, which favour economy of fuel, not promotion of vigorous health.

A. H. Barker calculates that for central heating in closed rooms the heat of one pound of coal would keep 1 sq. ft. of window warm for 3 weeks, of 9 in. wall 9 weeks, of ceiling protected by an air-tight, independent roof 12 weeks.

For open fires these figures must, he says, be divided by 6.

He says, if hospitals want to give patients 5,000 cu. ft. of air per hour and maintain the temperature of the ward, this must cost them at least half a ton of coal per patient per season. In my opinion the patients need radiant heat from a fire and cool air.

A. H. Barker gives the relative cost of the best house coal burnt in the best open modern grate sunk in wall as about one-third of the worst grate. Mrs. Fishenden found very little difference in the total radiation from modern or old types of grates for the same amount of coal burnt. It is the draught that makes the difference to the room temperature. The heat lost by the flue rises from 16 per cent. with one change of air per hour to 50 per cent. with 10 changes of air. The greater ventilation is good for health but uneconomical.

It is obvious that centralized heating applied to the new housing schemes may effect great economy of fuel and prevent smoke. Each house would be supplied with hot water for baths, wash-tubs, and radiators from a central station through well-insulated pipes. The cooking stove will be a gas one, and a gas fire ought to be provided in the sitting-room to give radiant heat. Central heating giving

[1] 'Fuel Economy,' Board of Trade leaflet, prepared by Instit. of Heating and Ventil. Engineers.

a monotonous, over-warm, still atmosphere will not promote health in people who have no opportunities for open-air exercise and do not recognize the need of such.

With the distribution of electric power and light, of hot water, and gas for heating, people need no longer crowd into tenement dwellings and pay exorbitant rents for vile conditions in order to be near their work. Home industry and home life can be revived.

A. H. Barker has warmed the floor of a room by radiant heat from an electrical source placed in the ceiling, the convected heat being removed by a flue. Ideal warm, fresh conditions are thus obtained, but the cost is prohibitive. W. A. Bone's compressed gas radiator may possibly be adapted to this end.

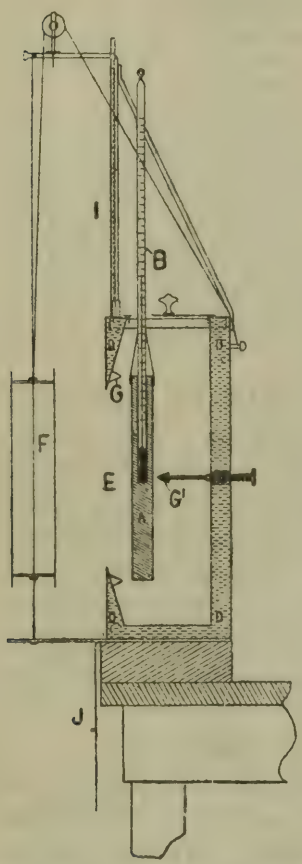


Fig. 49. The Richmond radiometer. Hartley.

Radiation from open or gas fires can be determined by means of the Bone-Callendar-Yates, or B.C.Y., bolometer or by the Richmond radiometer.

The bolometer[1] offers a small absorbing surface (about 2·89 in. sq.) of hard black enamel covering a coil of platinum wire wound on a thin mica plate. The increase of electrical resistance of the coil on exposure to radiation is measured (this can be done in a few minutes), and from this can be determined the intensity of the radiation per sq. ft. per hour. From measurements taken at the centre and in four other positions (at angular distances of 60° N. and S., and of 60° E. and W. of the central position respectively) of the hemisphere in front of a fire, the total radiation over the whole hemisphere may be calculated.

The radiometer designed by H. Hartley consists of a rectangular block of brass one face of which is corrugated and covered with lampblack. This block is suspended in the enclosure formed by a double polished metal case, and maintained at a steady temperature by the circulation of a stream of water through the latter. The case is provided with an opening 6 inches by 6 inches, through which the blackened face of the brass block is exposed to the source of radiation. A small hole drilled in the block along a vertical axis and filled with mercury to ensure sufficient heat contact contains the thermometer bulb, the projecting stem of the thermometer being shielded by a vertical strip of polished metal. Diagram—A is the metal block, B the thermometer in mercury, D the water jacket, E the opening 6 inches by 6 inches, F a double screen, and I the thermometer shield.

The apparatus is placed in position opposite the centre of the fire under examination, the absorbing block being protected by means of a double screen of polished metal. Temperature readings are taken

[1] Bone, Callendar, and Yates, *Proc. Roy. Soc.*, 1915, **A. 91**, 245.

at suitable intervals, alternately with the screen in position and removed. The latter readings give the rise of temperature of the block due to the radiant energy absorbed from the fire, the former the correction which must be applied to compensate for the transmission of heat from the block to the cooler surroundings.

The radiation from a modern open-grate coal fire, stoked at equal intervals, passes through cyclic changes, rising from a minimum immediately after coal is added to a maximum when the coal blazes most fiercely. Mrs. Fishenden finds the radiant efficiency, using coal, is 26 per cent. with sloping back, 21 per cent. with the firebrick back built up vertically. The efficiency is 24½ per cent. for dried coke and 19½ for coke with 16 per cent. moisture, burned in the fire with sloping back. 'Low temperature' coke yields 31–34 per cent. The maximum intensity of radiation is nearly at right angles to the surface. Allowing 5 per cent. for additional heat convected into the room, we see a well-stoked fire gives out about 30 per cent. of the energy of the fuel. The remainder passes up the chimney and helps somewhat to warm the house. If architects and builders placed chimneys on inner in place of outer walls more of this heat would be conserved. It is claimed that 60–70 per cent. of the energy of the fuel may go to warm the room and fabric of the house.[1]

The coal fire is a good ventilator, venting 18,000–20,000 cu. ft. of air per hour; it is variable—not monotonous—and cheerful to look at, and for a given output of heat the first cost of coal is only about 40 per cent. of gas (Bone).

A ten-inch gas fire working on London gas at 1·9 in. pressure and taking 24¼ cu. ft. per hour (at N.T.P.) gave a mean radiant efficiency of 45 per cent.[2] (43·2–46·6).

The convected heat obtained from the gas fire according to Yates was 21 per cent. of the thermal energy of the gas; the rest is flue loss, but some of this may go to warm house walls and upper rooms on the way up the flue.

Hartley gives the gross heating effect as less than 60 per cent., the convected heat being generally less than 10 per cent.

Gas fires have been greatly improved by the scientific researches of H. Y. Yates and others (1) in increase in radiant efficiency; (2) in removal of products of combustion by proper arrangement of canopy and flue outlet, (*a*) without the aid of any chimney pull, (*b*) without loss of radiant efficiency on increased chimney pull; (3) in silence.

The gas fire is not such a good ventilator as the open fire, and is monotonous. The attention a coal fire requires gives some measure of change and exercise to a sedentary person.

H. Y. Yates[3] has added an additional outlet to the gas fire flue so that the hot gases passing this act on the injector principle and carry up the flue a large volume of room air. The ordinary gas fire carried away 4,500–5,600 cu. ft. per hour, the new arrangement 11,800 cu. ft.

For rooms occupied all day a coal fire is cheaper; for rooms occupied for short periods a gas fire.

The poor, who can afford to keep going one fire only for warmth

[1] *Rep. Fuel Research Board*, 1918–19, *Department of Scientific and Industrial Research.*
[2] Bone, Callendar, and Yates, *Proc. Roy. Soc.*, 1915, **A. 91**, 245.
[3] *Engineering*, Oct. 15, 1915.

and cooking, naturally choose coal in the winter; in the summer a gas cooker may be most economical for them, using coal for the boiler on wash day.

To get warmth and conserve fuel and food the poor naturally shut up their dwellings and crowd in one room. Poverty is the cause of unhygienic conditions.

The great desideratum is the cheapening of gas, and the use of a smokeless coke or semi-coke in place of coal. The preparation of gas or smokeless fuel involves expenditure in wages, &c., which may amount to one-third of the value of the coal.

Professor W. A. Bone estimates that it is possible to purchase in the form of coal about five times the number of potential thermal units purchased as gas, and about twenty-five times those purchased in the form of electricity.

Taking into account, however, the great disparity between the relative 'overall' efficiencies of the best heating appliances, using coal, gas, or electricity respectively, the corresponding ratio between the amounts of 'utilized' energy purchased for a given sum of money would, he says, be approximately 10 : 4 : 1. Electricity for heating is, then, the luxury of the rich, and the question of gas or coal is one of convenience and cleanliness and continuity of use.

Mrs. Fishenden found that the fire in a modern grate threw its most intense radiant heat upwards, the maximum being at an angle of about 57° to the horizontal plane through the centre of the fire. The horizontal distribution showed a maximum in the vertical plane through the centre of the fire and fell off continuously to the sides.

With a large draught aperture the fire burns up quickly, and goes low before the next stoking. With small aperture the radiation is far more steady, but given a grate and a fixed weight of coal to be burned, the total radiation received varies little with the speed or manner of burning.

The form and construction of the grate, whether with sloping or vertical back, whether mostly made of iron or firebrick, as far as total radiant efficiency goes is of no importance according to Mrs. Fishenden.

The modern fire-grate, barless, with raised hearth and sloping firebrick back, gave an efficiency of 26 per cent. The following two grates gave efficiency of 24–25 per cent.:

B. A very large old-fashioned grate with vertical firebrick back and sides, and a base of metal grid $10\frac{1}{2}''$ from back to front, and $28''$ wide, with five iron bars in front, each $\frac{1}{2}''$ thick and $2\frac{1}{2}''$ apart.

C, A small old-fashioned register grate $7''$ only from back to front and $14''$ long, with four $\frac{1}{4}''$ iron bars $1\frac{1}{2}''$ apart. The canopy and sides of the grate of iron, the base a horizontal iron grating. A small vertical firebrick back.

There is nothing, then, as far as regards total radiant efficiency in the claims for greater efficiency of fire-grates designed according to the principles laid down by Teale, viz. grates shallow, wide from back to front, with few or no bars, sides and back of firebrick, and back inclined forwards to minimize loss of radiant heat up the chimney.

The old Adams fire-place with graceful lines and wide hobs is as efficient as beautiful. The grate must be elevated above the level of the floor, so that the radiant heat is projected horizontally and warms

the floor. Fires on a level or sunk below the floor are wrong in design, for they do not warm the most essential part of the body—the feet.

The radiant heat from a fire should warm the lower part of a room, not the ceiling.

Vernon Harcourt justly recommended a form of grate which conforms to the following requirements :

(1) That the face of a fire should be vertical so that the chief radiation should be horizontal.

(2) That the bars should be as light as is compatible with sufficient strength, and wide apart, for they do not reach so high a temperature as the glowing fuel behind.

(3) The fire should be narrow from back to front.

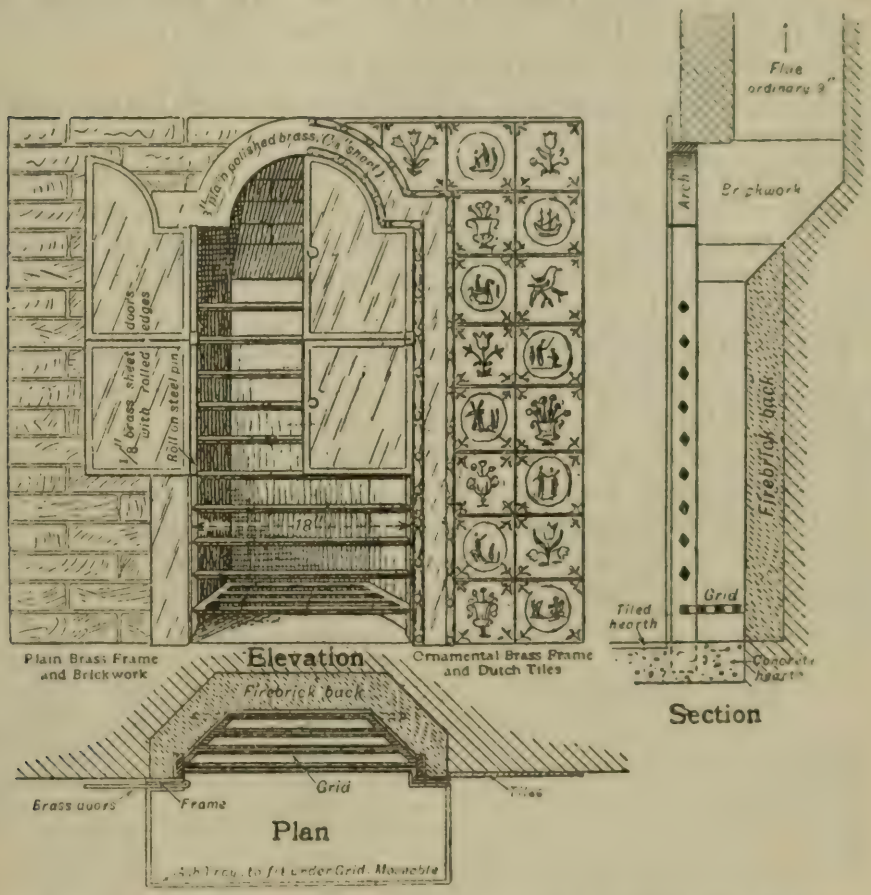


Fig. 50. Fire-grate for burning coke. Vernon Harcourt and Kitchin.

(4) Air should pass in through bars in front, not through bars underneath, for the most vivid combustion occurs where the air and fuel meet.

It is well, he says, to put in at once all the fuel the fire will need and to light it at the top.

The doors for regulating the draught are of great value. By their use escape of fumes is prevented. It must be remembered that, under certain conditions of calm and high barometer, the up-pull of a chimney may become nil or negative.

Comparative figures for the radiation from coal and coke fires with such a grate were as follows : coke, 1·97 ; coal, 1·63 ; wet coke, 1·29.

He says, ' Bulk for bulk coal is nearly twice as heavy as coke, burns

for longer under the same conditions, produces more heat, and from an open fire radiates rather more heat because it lasts longer.'

'Weight for weight, coke has the advantage for domestic use, and would retain it even if the price of the two were the same.'

With this design of grate the fire was, after the first hour or so, practically smokeless, even when burning ordinary bituminous coal. By that time the top layer of coal had been converted into a glowing layer of coke, through which the soot and gases from the combustion of the lower layers had to pass on their way to the chimney. All combustible parts were therefore burned, and the fire was then smokeless.

Mrs. Fishenden proved that the salts widely advertised for spraying on coal as effecting economy in its use have no effect at all on the total radiation. They consist of a little ferric oxide and salts of magnesium and sodium, chiefly common salt.

W. A. Bone points out that fuel can be conserved by not throwing open windows in the chilly early morning hours, but in the middle of the day when the sun is out or the air warmer. There is pleasure, however, in coming down to a cool room with tobacco smoke blown out of it. In the case of central heating a wise plan is to warm up the rooms by night, and then open the window and enjoy the radiant heat from warm walls and cool air.

In one of Mrs. Fishenden's observations the grate was raised 34·5″ above floor-level, and the fire was lighted with 15 lb. of coal and 0·5 lb. of wood, 7·5 lb. of coal being added every hour.

The table given below shows the radiation in kilocalories per sq. foot per hour secured over one square foot area 34·5″ from the centre front of the fire in successive intervals between stoking. For comparison with heat loss of kata these values have been converted into millicalories per sq. cm. per minute, and the results given in the third column :

After lighting fire	Kilocalories per sq. ft. per hour	Millicalories per sq. cm. per minute
1st hour	37	666
2nd ,,	94	1,692
3rd ,,	172	3,096
4th ,,	188	3,384
5th ,,	185	3,330
No more coal added		
6th ,,	196	3,528
7th ,,	207	3,726
8th ,,	149	2,682
9th ,,	53	954
10th ,,	15	270
11th ,,	3·5	6·6
Total radiation from lighting to dying out of fire	1,299	23,382
Calorific value of fuel burned	209,000	
Efficiency	25·5 per cent.	

From the above table the mean value of the radiant heat for the six hours from the third hour to the eighth hour after lighting the fire is 3,292 millicalories per sq. cm. per minute at a distance of 0·876 metres (34·5″) from the centre front of the fire.

The figures given below, from which graph is plotted, were obtained

by Miss Hargood Ash on the assumption that the distribution of radiant heat follows the inverse square law, i.e. if H_1 is the heat at a distance r_1 and H_2 the heat at a distance r_2 from the fire, then

$$H_1 = \left(\frac{r_2}{r_1}\right)^2 H_2.$$

Distance from fire metres	Heat Millicalories per sq. cm. per minute
0·876	3,291
1	2,525
1·5	1,122
2	631
3	281
4	158
5	101
6	72

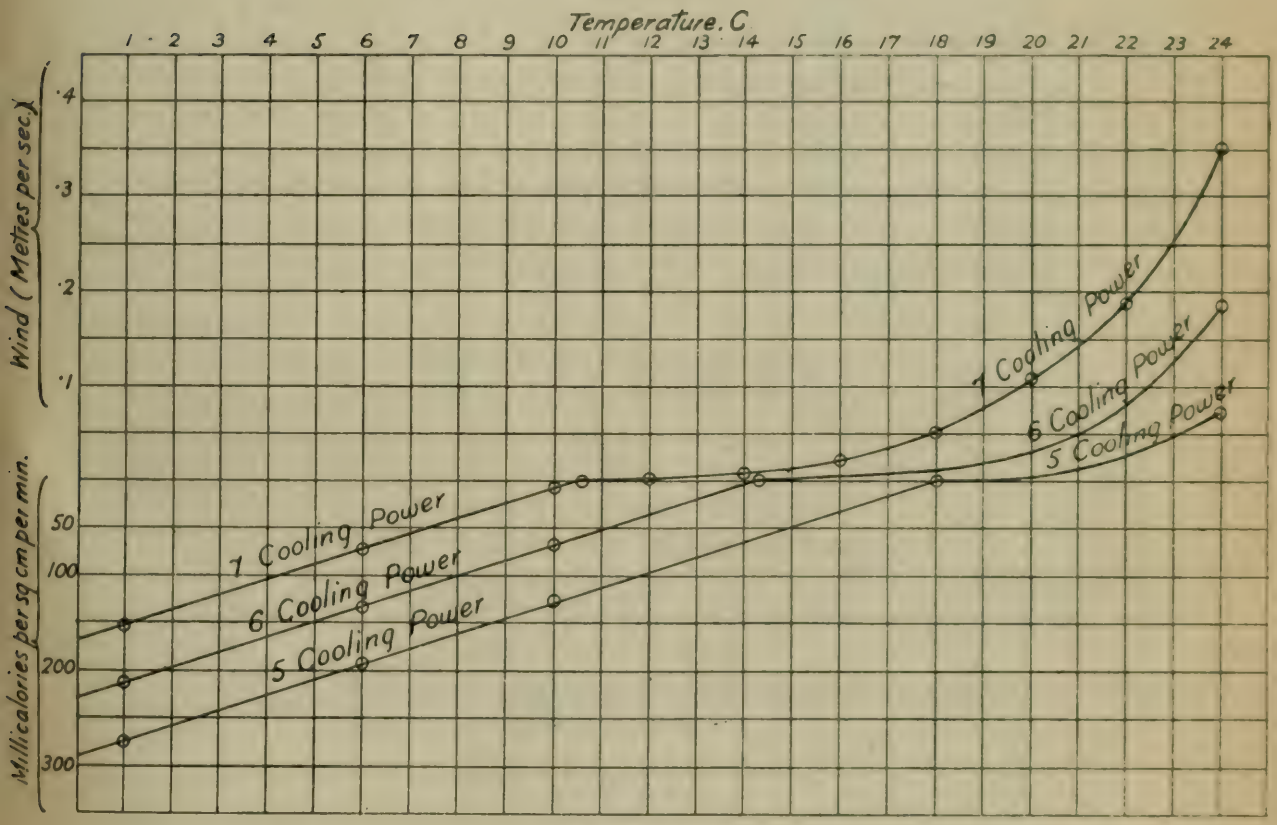


Fig. 51.

Six or seven is a comfortable dry kata cooling power for sedentary workers. Now any given cooling power may be obtained in still air at one temperature only. To obtain the same cooling power at higher temperatures than this the kata must be exposed to a current of air, and at lower temperatures to radiant heat. Fig. 51 shows (*a*) the wind velocity in metres per sec. necessary to produce a cooling power of 5, 6, or 7 at temperatures up, say, to 24° C.; (*b*) the radiant heat in millicalories which the kata must absorb per minute to give the cooling power 5, 6, or 7 for temperatures down to 0° C.

Examples from graph:

(1) To obtain a cooling power of 7 at a temperature of 21° C. requires a wind of 0·140 metres per sec.

(2) To obtain a cooling power of 6 at a temperature of 7° C. the kata must absorb 118 millicalories of radiant heat per minute.

Assuming that the kata absorbs all the radiant heat which reaches it (and neglecting its curvature of surface) then from Fig. 52 may be determined the heat in millicalories which is absorbed per minute from source of heat which is at a distance 1, 2, 3, 4, 5, or 6 metres from the kata, supposing that to obtain a cooling power of 6 at a temperature of 7° C. the kata at a distance of 6 metres receives from the source of heat 118 millicalories per sq. cm. per minute ; as, however, only one-half of the kata is exposed to the radiation this amount must be doubled, and the required heat per minute will be 252 millicalories.

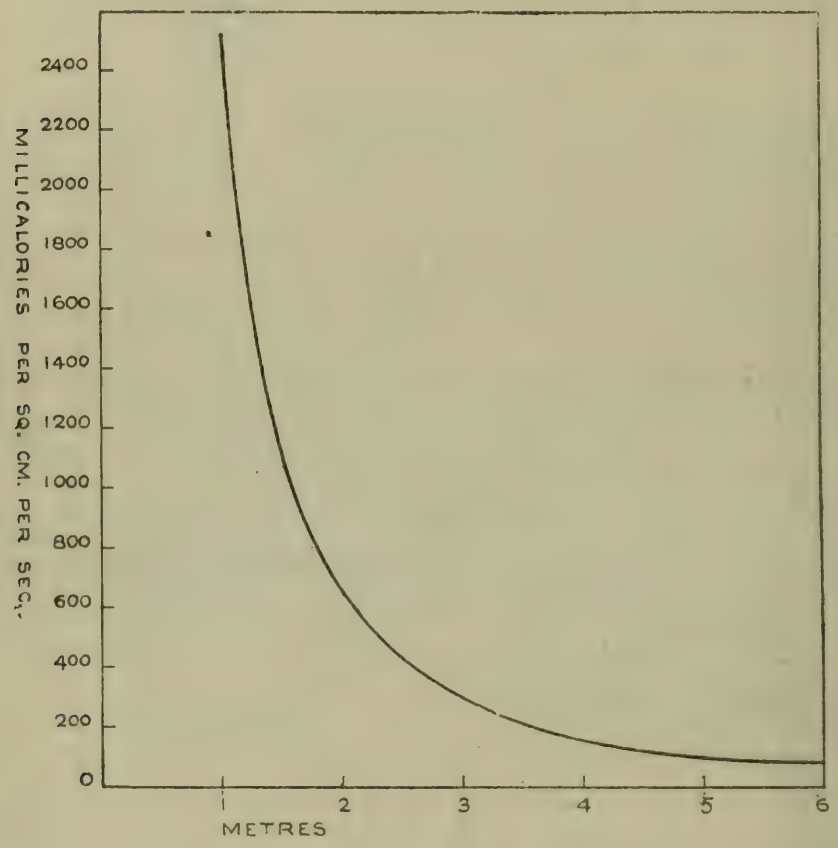


Fig. 52.

Fig. 52 shows this amount of heat will be obtained 3·25 metres from the centre of the fire.

As the heat of combustion of coal (average)

= 8 calories per grm.

= 406,416 calories per cwt.,

then $\frac{252 \times 60}{406,164} = 0{\cdot}037$ cwt. of coal per hour, i.e. the coal must be burnt at the rate of 1 cwt. in 27 hours.

The above assumes that the source is radiating out heat equally in all directions, and that the total heat of combustion is lost as radiant heat. In the case of an ordinary fire the heat from the back must to a certain extent be reflected to the front, and this is not taken account of in the above calculation.

Since it is assumed that the kata absorbs all the radiation which reaches it, the amount as calculated from the graphs must be the minimal quantity.

Assuming that one-fifth only of the fuel energy of the coal is obtained as radiant heat, and that the dry kata-thermometer cools four or five times as quickly as the clothed man at rest in a room, the rough estimate of $\frac{1}{2}$ cwt. for 27 hours holds good for the man, or 28 lb. of coal per working day when the air temperature of the room is 7° C. As pointed out above, to save coal and secure comfort the natural tendency is to shut down ventilation and raise the air temperature.

The graph also shows how the radiant heat rapidly falls away on removing from a fire, and therefore how defective a wall fire may be for warming pupils in a school or clerks in an office. It must be remembered that any solid obstruction screens one from radiant heat. Those, then, near the fire are too hot and those far away too cold. The charm of an open fire depends on freedom of the individual to move to and fro and warm himself at it or go back to his desk to work.

In the case of a large schoolroom no single gas or coal fire can supply the radiant heat to warm the whole room on a winter's day, but if hot-water radiators are fixed below the windows these can warm up the building during the night and then be turned off, except in exceptionally cold weather, when the room is occupied, the fire being kept to give the students radiant heat in the intervals and to aid in ventilation.

To make a gas fire efficient in a school or office it should be spherical in form, and the desks arranged round it so that each shares in the radiant heat. Three or four such fires might be placed in a large room, each with the desks in a circle round it. By such means each of the pupils or clerks might secure radiant heat.

As suggested above in the case of central heating, hot-water coils or hot air might be best arranged to warm the floor so that the feet are kept warm, not the head. With warm feet cool air would be tolerated.

Such an installation has been provided in the new wards of the Derby County Sanatorium by Dr. Barwise.

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MEDICAL RESEARCH COUNCIL

THE MEDICAL PROBLEMS OF FLYING

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MEDICAL RESEARCH COUNCIL

THE MEDICAL PROBLEMS OF FLYING

Including Reports Nos. I–VII of the Air Medical Investigation Committee



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Sydney Scott, F.R.C.S.
Professor C. E. Spearman, Ph.D.
Lieut.-Colonel Martin Flack, C.B.E., M.B., R.A.F. (*Secretary*).

The following have also served upon the Committee at earlier periods during the war:

Sir Walter M. Fletcher, K.B.E., M.D., F.R.S.
M. Greenwood, M.R.C.P., M.R.C.S. (late Capt. R.A.M.C.).
Professor C. S. Sherrington, M.D., F.R.S.

INTRODUCTION

During the course of the war, and with successive improvements in artillery, flying was gradually carried to greater and greater heights, so that by the autumn of 1916 'mountain sickness' in its various kinds and degrees gravely increased the difficulties and dangers met by flying officers, and the practical problems of the administration of oxygen at high altitudes by suitable methods had vital military importance. It became apparent also that manifold physiological problems of other kinds were confronting the medical officers in the separate Flying Services who were responsible for the selection of suitable candidates for commissions, for the maintenance of efficiency in the flying men and their restoration after fatigue or damage. During the later months of 1916, the Medical Research Committee had given assistance in France and at home to experimental work undertaken by individual medical officers or civilian physiologists, by supplying special apparatus and in other ways. At the close of that year the Committee formally offered their services in these matters to the President of the Air Board, and supplied some further expert physiological assistance to the experiments and investigations already begun under the Directorate of Military Aeronautics. Early in 1917 an Air Board Research Committee (Medical), which included the Secretary of the Medical Research Committee, was formed to deal with these physiological questions and with other medical problems of aviation, with a view to bringing together more effectively the scientific work being done for the then separate Flying Services, whether in the Navy Medical Department, the Army Medical Service in France, for the Air Board at home, or in University and other laboratories. In particular, the Medical Research Committee gave financial and other assistance to work aimed at finding the most effective means of storing and carrying oxygen in the air and the best method of its convenient administration to flying men. In this country, by permission of the Director-General, A.M.S., the services of Captain J. G. Priestley were provided to follow the lines of earlier work by Dr. J. S. Haldane upon the methods of oxygen administration, and Captain Priestley, in touch with the work being done by Dr. Haldane in the Navy Medical Department, supervised the immediate provision of emergency sets of oxygen apparatus. In France, Professor G. Dreyer (then Major, R.A.M.C.) turned from other work in hand for the Committee, and was afforded facilities for the perfection of an oxygen apparatus devised by himself, which eventually came into general use. In March 1917 the services of Lieut.-Colonel Martin Flack, R.A.F. (then Captain, R.A.M.C.), a member of the scientific staff of the Committee, were placed at the disposal of the Air Board. Lieut.-Colonel Flack dealt in general with the question of oxygen administration and the provision of oxygen apparatus in this country. He began the organization of further studies directed towards finding the most effective tests to be applied to candidates for commissions and detecting the physiological after-results of flying fatigue and strain. For his assistance the services of Dr. Grace Briscoe were placed by the Committee at the disposal of the Air Board for special work at the Central Hospital for Flying Officers established in the building at Hampstead, lent then by the Medical Research Committee to the War Office and later to the Royal Air Force at Hampstead.

In the autumn of 1917, with the prospect of the fusion of the Royal Naval Air Service and the Royal Flying Corps, a new Committee, the Flying Services Medical Advisory Committee, was formed, a sub-committee of which dealt with the questions of medical research. This Committee, in turn, was superseded by the Medical Administrative Committee of the Royal Air Force when that Service was separately established. On both these Committees the Medical Research Committee was directly represented.

In March 1918 the Medical Administrative Committee, in consultation with the Medical Research Committee and with the concurrence of the Air Council, appointed an Air Medical Investigation Committee, (*a*) to

(6555) Ps. 4024. Wt. 42879. 286/457. 1750. 8/20. O.U.P.

prepare reports for submission to them with a view to their distribution to those concerned, (*b*) to make suggestions for further co-ordinated medical investigations that might seem desirable, (*c*) to advise them generally in respect of the scientific medical aspects of aeronautics. With the discharge of the Medical Administrative Committee in November 1918 the Medical Research Committee, in consultation with the Director of Medical Services, R.A.F., made arrangements to continue the scientific work of the Air Medical Investigation Committee, and re-appointed it with some modifications in its constitution.

From the date of its inception, the Air Medical Investigation Committee has received and prepared for issue a series of reports upon the scientific work done in this subject. These have been circulated widely through official channels among the medical officers serving with the Flying Services, both at home and abroad, and among officers and others interested in the medical problems of flying in Allied countries.

With the relaxation of censorship regulations after the Armistice, two further reports, respectively by Major Kellaway, M.C., and Major Sydney Scott, were published in the present series (No. 37) by the Medical Research Committee (now the Medical Research Council). It has now been thought desirable that the earlier reports, which contained work of much scientific and medical interest, should be made generally available by publication, and they are here brought together with such additions and emendations as their authors have desired for the inclusion of more recent observations. Many of the tests described in this volume are now in routine use in the Royal Air Force, and the results have already shown that they are valid for the purposes in view.

These various reports are now arranged, not in the order of their former issue during the war, but so as to present in better sequence the problems studied. Additional reports by Dr. Grace Briscoe, the late Dr. L. E. Stamm, and Dr. Rivers and Major Rippon are also now included.

The Medical Research Council are heavily indebted to the Air Medical Investigation Committee for the care they have given to this compilation, and no less to the individual authors who have presented here the results of their work. They would take this occasion of referring with the deepest regret to the untimely deaths of Dr. Corbett and Dr. Stamm, who have contributed to this volume. While connected with the Air Force Medical Service both these officers showed the keenest interest in the study of the medical problems of flying and their loss to the Service and to medical science is very great and widely felt.

The Council have greatly valued the abundant opportunities continuously offered them by the Air Council and by the earlier authorities responsible at various stages for the development of the Flying Services. These opportunities provided a field of work of very great physiological and medical interest. The scientific work actually done during the war not only gave results at the time of immediate military value, but opened up many questions of general medical importance, from the further study of which far-reaching benefits may come. It will not be necessary for the Council to point out that in these present reports they are offering the results of work which, carried out under the trying conditions of war and often in conjunction with heavy duties of a routine kind, has been of necessity in some measure incomplete. It is hoped that knowledge in this subject may be placed upon a more and more trustworthy basis. In the meantime it is believed that the present compilation will give valuable information to all who are interested in the medical problems of flying, and that some of the methods described will be found to give service in medical work wholly unconnected with flying.

Medical Research Council,
15 Buckingham Street,
Strand, London, W.C. 2.

May 10, 1920.

THE MEDICAL PROBLEMS OF FLYING

CONTENTS

PAGE

1. Report on the Medical Aspects of High Flying, by J. L. Birley, C.B.E., F.R.C.P. 5
2. A Simple Procedure for Testing the Effects of 'Oxygen Want' on Flying Men, by Professor G. Dreyer, C.B.E. 10
3. The Bag Method for the Investigation of Air Disabilities of Aviators, by Lieut.-Colonel Martin Flack, C.B.E. 14
4. A Study of the Reaction of Pilots and Observers to Diminished Oxygen-pressure, by the late C. D. H. Corbett, M.D., and H. C. Bazett, O.B.E., M.C., M.B., F.R.C.S. 18
5. A Report on the Value of Oxygen to Aviators at relatively Low Altitudes, by Lieut.-Colonel Martin Flack, C.B.E., and Lieut.-Colonel C. B. Heald, C.B.E. 70
6. The Selection of Candidates for Flying, by Lieut.-Colonel Martin Flack, C.B.E., and Lieut.-Colonel A. P. Bowdler, O.B.E. 81
7. Tests for Flying Efficiency and Flying Strain, by Lieut.-Colonel Martin Flack, C.B.E. 93
8. Temperament and Service Flying, by J. L. Birley, C.B.E., F.R.C.P. . 141
9. Respiratory Tests for Ability to stand High Altitudes, by H. C. Bazett, O.B.E., M.C., M.B., F.R.C.S. 184
10. Observations on the Respiration of Airmen, by Grace Briscoe, M.B., B.S. 203
11. The Sense of Stability and Balance in the Air, by Henry Head, M.D., F.R.S. 214
12. Mental Aptitude for Aviation, by W. H. R. Rivers, M.D., F.R.S., and Squadron-Leader T. S. Rippon, O.B.E., R.A.F.M.S., in collaboration with the late Lieut. E. G. Manuel, R.A.F. 257
13. 'Reaction Time' Tests carried out on Flight Cadets, with description of Apparatus, by the late L. E. Stamm, M.D. 265

1.—REPORT ON THE MEDICAL ASPECTS OF HIGH FLYING

BY

J. L. Birley, C.B.E., F.R.C.P., late Lieutenant-Colonel R.A.F. and Medical Officer in Charge, R.A.F., in the Field.

(*First printed March* 23, 1918. *Revised February* 1920.)

A. Preliminary.

It is clearly impossible to draw an absolutely sharp line of distinction between the immediate and remote effects of flying at low as opposed to high altitudes, because many symptoms are common to both types. At the same time, in healthy and fit individuals who fly from 15,000 to 20,000 feet or more, a group of symptoms can be

isolated which may properly be attributed to the physical effects induced by the lowered pressure of oxygen in the air breathed.

It must be borne in mind that the necessity of attention to the technical and other duties of a flight may, in itself, suffice to divert an officer's notice from physical symptoms, unless the latter become imperative, and, further, that one of the most important effects of oxygen starvation is a dulling of the judgement and intellect and an unwarranted sense of well-being and security. In this connexion, the cases of two flight-commanders may be cited who averred they were just as fit at 20,000 feet as on the ground, and scoffed at the idea of using oxygen, while a week later, having both used oxygen for a long flight of more than three hours over 18,000 feet, they were loud in their praises of the benefits it conferred, and made its use compulsory among their pilots and observers.

The most interesting aspect of the whole question, and one of great practical importance, is the remarkable variation met with among individuals who are apparently equally healthy. The use of Flack's bag experiment (see Report 3, this volume) enables one to isolate those very rare cases of men with heart and lungs stethoscopically normal who are so peculiarly sensitive to the effects of even a moderate fall of barometric pressure that they are practically debarred from flying of any description. But beyond these quite exceptional cases, marked differences exist as regards the reaction of the organism to diminished oxygen pressures, and many pilots, especially scout pilots, notice nothing unusual when flying at 18,000 feet.

It should be pointed out that flying at or above 18,000 feet was, in the earlier stages of the war, the exception rather than the rule. The comparative immunity which scout pilots certainly enjoy is largely explained by the fact that they do not maintain great altitudes for very long, and that the actual fighting, with very few exceptions, took place at 15,000 feet or very much lower.

B. Symptoms Noticeable while in the Air.

(*a*) *Dyspnoea.*—This is quite common, and very few pilots breathe through the nose above 12,000 feet. It is often described as ' an inability to fill the lungs with air '. Large numbers of pilots have given me accurate descriptions of periodic (Cheyne-Stokes) breathing at great heights, such as : ' I suddenly begin to gasp for breath, and then do not seem to want any more for a minute or two.'

The slightest exertion brings on dyspnoea, and this is particularly noticeable among observers when fighting, taking photographs, &c. The amount of work involved in fighting is considerably greater than is sometimes supposed.

I have personally experienced Cheyne-Stokes breathing at 20,000 feet, which was at once relieved by breathing oxygen.

The deep breathing persists as long as the height is maintained, but as a rule only causes inconvenience during or after exertion. Pilots being more or less immobilized are much less affected, but there have been cases of pilots arriving back at the aerodrome thoroughly exhausted after having had to use their pressure pump at high altitudes.

(*b*) *Muscular Weakness.*—This is complained of chiefly by observers. For example, bending down and drawing the shutter of a camera is often ' hard work '. Several observers have had to break off an engagement even at comparatively low altitudes (e.g. 14,000 feet) owing to exhaustion brought about by standing up in the cockpit and swinging the gun round on its mounting.

I was personally unable to stand up in the observer's seat after being at 21,000 feet for twenty minutes.

(*c*) *Impairment of Judgement and Perception.*—This certainly occurs in the great majority of individuals, but frequently passes unnoticed.

The following facts are of interest :

A pilot in a Bristol ' Fighter ' met a patrol of 5 E.A. (Enemy Aircraft) at 18,000 feet. He did not realize his danger, but waved his hand to them in spite of the protests of his observer.

Some officers doing long reconnaissances experience difficulty in recognizing the country over which they are flying. As one officer expressed it : ' I could see the ground quite well, but could not puzzle out where I was.'

Observers on high photographic reconnaissances frequently get muddled and fog their plates. They are often unaware of their mistakes, and indignantly attribute the bad results to some fault of the camera. The routine use of oxygen has practically abolished this trouble in one squadron which does a lot of this kind of work.

Pilots who have been up to 20,000 feet have more than once come back with reports of having seen formations of E.A. 4,000 or 5,000 feet above them. It is practically certain that their observations were not correct.

The inefficiency which overcomes pilots and observers at great altitudes is frequently not remarked on by them owing to dulling of perception. This is a most important point, and one that requires systematic investigation both in the laboratory and in the air. At great altitudes there is either a general slackening of morale and loss of offensive spirit, or else a feebleness of judgement which may land a pilot into unnecessary difficulties. In fact, the effects of lack of oxygen on the brain are infinitely more important than the more obvious effects such as hyperpnoea, &c., and constitute for the pilot a subtle and insidious danger of the existence of which it is in many cases impossible to convince him.

(*d*) *Syncope.*—It is, in my opinion, a grave mistake to attribute every case of syncope in the air to the lowering of oxygen pressure alone. In very many instances this is only one of the determining factors, and more than 50 per cent. of such cases which have come to my knowledge have occurred below 10,000 feet. Nevertheless there are instances in which the fall of barometric pressure is the main and perhaps the only cause, and, in such, loss of consciousness usually comes on slowly and persists for a relatively long time. In fact, the condition is more like falling to sleep, and I know of a few pilots who state that they have fainted at big heights and cannot remember landing, whereas they have actually been sufficiently awake to fly the machine and land it on their own aerodrome with verbal assistance from the observer.

The following case may be cited :

A squadron-commander was returning from a long bomb raid at 17,000 feet, using oxygen. As he did not begin to descend after crossing our lines his observer roused him and discovered that he had been dozing, his oxygen supply having failed.

Sudden syncope sometimes occurs among observers after making any physical effort, and also occasionally when getting out of the machine.

(*e*) *Overfilling of the Bladder.*—Nearly all officers engaged on long patrols at 17,000 feet or over complain of this, and many of them have to answer the calls of nature while in the air as best they can. This is probably accounted for by the combination of cold and vibration.

(*f*) *Frontal Headache.*—This sometimes comes on while in the air, but more usually after landing.

(*g*) *Vomiting.*—This is very rare indeed.

(*h*) *Haemorrhage from Mucous Membranes.*—This is practically unknown.

C. Symptoms coming on After Landing.

(*a*) *Fatigue.*—This is the most universal and, from a practical point of view, the most important after-result of high flying. All flying is fatiguing owing to the constant stream of stimuli which are pouring in, as, for example, those impinging on the external ear, the internal ear, the eye, the higher centres, muscles, skin, &c. Moreover, many of these are of a character to which the organism is comparatively unaccustomed.

It is only natural that the effects produced when the blood is insufficiently oxygenated should be magnified, and one only has to watch a flight landing after a two hours' patrol without oxygen at 18,000 feet (perhaps the second in the day) to realize the enormous strain which modern flying under active service conditions imposes. Reports of the patrol are laboriously made out at the squadron office, and then there is a general movement to lie down and go to sleep. The gait is often unsteady and laboured, and the general sense of lassitude and fatigue is reflected in the face and general demeanour.

(*b*) *Frontal Headache.*—This comes on shortly after landing, and persists for two hours up to twenty-four or more. At times it is sufficiently severe to render a man incapable of flying.

(*c*) *Cardio-Vascular System.*—Pilots occasionally land with small pulses and low-blood pressures, but observations made on aerodromes on active service are both inopportune and misleading.

(*d*) *Respiratory System.*—Shortness of breath and inability to hold the breath for more than twenty to thirty seconds persist for half an hour or so.

D. Acclimatization.

Many flying officers state that they get accustomed to high altitudes. In my opinion this merely means that they get used to the symptoms thereby induced, and there is no evidence that acclimatization in the true sense of the word occurs to a sufficient extent as to render it of any practical utility.

E. Practical Considerations.

Suitable clothing has produced a very noticeable amelioration of the immediate ill effects caused by high altitudes. One squadron has recently been carrying out long bomb raids at 15,000 feet without oxygen, but well equipped as regards clothing, and their pilots have returned to the aerodrome in good condition and with comparatively little fatigue. This is not surprising when the great increase in the respiratory exchange from exposure to cold is borne in mind.

It is also interesting to remember that cold is an extremely important etiological factor in the production of surgical shock, a condition which is closely allied to the condition induced by extreme lack of oxygen in the healthy organism.

The unpleasant effects of flying at great heights are closely dependent on the number of hours flown in a unit of time, and 50 hours high flying in three weeks is productive of greater strain than 100 hours in three months. In other words, the organism must be given time to recover.

Another factor of great practical importance is the length of exposure to heights such as 18,000 feet, and the intervals between flights. Short exposures (scout pilots) are well borne, whereas longer ones are always productive of some of the above-mentioned symptoms. During the winter months, when so many days are impossible for high flying, and the hours are short, the number of breakdowns is reduced to a minimum, and for this happy state of affairs I consider that physical exercise on the ground, which is encouraged and popularized at this time of the year, is also partly responsible.

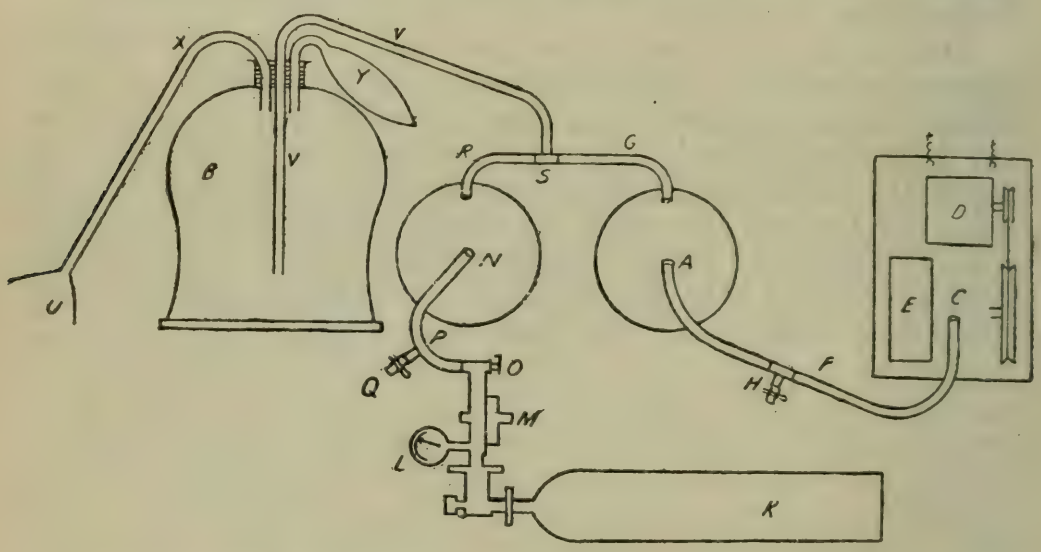
2.—A SIMPLE PROCEDURE FOR TESTING THE EFFECTS OF 'OXYGEN WANT' ON FLYING MEN

BY

Professor G. DREYER, C.B.E., late Lieutenant-Colonel, R.A.F.

(*First printed March* 23, 1918.)

FOR the study of the effects of 'oxygen want' on flying men, whether for selection or for the purpose of testing staleness, flying fatigue or other symptoms that may develop as a result of continued flying, particularly at high altitudes, it is important to make use of



a procedure that eliminates other complicating effects of a decreased atmospheric pressure on the organism.

For this purpose the following simple method has been devised. The arrangements are easily seen from the diagram.

A blower (C) (Down's) worked by a small electric motor (D), governed by a resistance (E), allows different quantities of atmospheric air to be passed through an accurate gas-meter (A) by means of a tube (F) of wide bore. From the gas-meter (A) ('air-meter') an exit tube (G) of similar bore is connected with a large container (B) ('breathing chamber') of a capacity of about 30 to 40 litres, preferably about 35 litres.

A T-piece (H) is inserted into the tube (F) connecting the blower with the air-meter. To the free end of the T-piece is fixed a short length of thick-walled india-rubber tubing of a small lumen, to which a screw-clip is attached. By this means a very fine regulation of the quantity of gas passing through the air-meter can be obtained, if a more delicate regulation than can be secured by the electrical resistance, is required. A nitrogen cylinder (K) of a capacity of about 1,000 to 1,500 litres (at 150 atmospheres pressure), and

provided with a manometer (L), a reduction valve (M) and, peripheral to the reduction valve, a stop-cock (O) allowing one to regulate the nitrogen delivery, is connected up by means of wide tubing (P) to a gas-meter (N) ('nitrogen meter') exactly similar to the air-meter. A T-piece (Q), fitted with thick-walled india-rubber tubing of a small lumen, and provided with a screw-clip for fine adjustment of the quantities of nitrogen passing through the nitrogen meter, is inserted into the tubing (P) leading from the nitrogen cylinder to the meter. The exit tube (R) from the nitrogen meter (N) is connected by means of a T-piece (S) to the exit tube (G) leading from the air-meter (A). The inlet tube (V), i.e. the joint exit tube from the two meters, should be passed near to the bottom of the breathing chamber.

The reduction valve made use of should allow quantities of nitrogen of not less than 16 litres per minute to pass through the meter, and the stop-cock should allow regulation of quantities from trace of gas up to a delivery of 16 litres per minute.

Into the breathing chamber is further fixed a wide bore exit tube (X), connected up with a face mask (U).

The mask that I have found preferable to others is the French 'Tizot' mask. It is a tight-fitting mask made of pure india-rubber, fitted with a very light 'knife' valve, arranged in such a manner that it, during inspiration, shuts off all connexion with the external air and allows the air from the breathing chamber to pass freely into the mask, while it, during expiration, shuts off connexion with the breathing chamber and ensures that the expired air shall pass into the external air, thus preventing the expired carbon dioxide from penetrating into the breathing chamber. To prevent the unpleasant and interfering effects of an even slight diminution in pressure caused in the breathing chamber with its rigid walls by each inspiration, an india-rubber bag (Y), for example, the ordinary india-rubber 'bladder' of a football, is fixed into the container.

By the above arrangement it is possible to let pure atmospheric air, or a mixture of atmospheric air and nitrogen, pass into the breathing chamber, and accordingly at will to change the relative proportions between atmospheric air and nitrogen; or, in other words, to change the partial pressure of oxygen at any desired rate, thus obtaining exactly the same effect as if the partial pressure of oxygen were reduced by mounting in the air, gradually arriving at a lower and lower barometric pressure.

The apparatus is used in the following manner:—The electric motor is started, allowing a quantity of about 22 to 27 litres of atmospheric air per minute to pass through the air-meter into the breathing chamber. At this time the T-piece (Q) inserted into the exit tube (P) leading from the nitrogen cylinder should be closed by means of the screw-clip. When the bag is more or less filled, then the person on whom the experiment is going to be carried out puts on the face mask, securing that it fits tightly without leakage. He is now allowed to breathe pure atmospheric air for some minutes. Under these conditions (normal conditions) all control examinations should be carried out—for example, frequency of pulse and respiration, haemoglobin percentage, reaction times, &c.

The tap of the nitrogen cylinder is then opened, and by means of the controlling valve increasing amounts of nitrogen gas are allowed to pass through the nitrogen meter. At the same time the amount of atmospheric air passing through the air-meter is gradually diminished in such a manner that the total volume of gas passing into the breathing chamber in each unit of time remains practically the same during the whole experiment. By this procedure it is possible to reduce the partial pressure of oxygen at any rate which is desired (for example, the rate at which it falls when mounting in a fast or medium-fast aeroplane), and to maintain any given pressure for any period that may be required, or suddenly to decrease or increase it, &c.

To secure accurate results it is obvious that the accuracy of the meters should be tested beforehand, and their individual error determined and allowed for in the subsequent calculation. For further control it is also advisable from time to time to make a gas analysis of a sample of the air mixture contained in the breathing chamber, or of the nitrogen used.

It is evident that if equal volumes of air and nitrogen pass through the two meters in the same unit of time, and are properly mixed, the partial pressure of oxygen in the mixture passing into the breathing chamber corresponds to an atmospheric pressure of half-normal or a barometric pressure of 380 mm.

The simplest way to calculate the barometric pressure corresponding to a given oxygen partial pressure in the mixture of air and nitrogen is to observe by means of a stop-watch the times taken by a given volume of air and nitrogen to pass through their respective meters. For instance, the number of seconds it takes for 1, 2, or 2·5 litres respectively. It is preferable to observe the time it takes for 2 or 2·5 litres to pass, because the error of experimentation becomes small or insignificant. If P represents the barometric pressure at the time of the experiment, and T_n the time it takes for a given quantity (for example, $2\frac{1}{2}$ litres) of nitrogen to pass through the meter, and T_a the time it takes for the same quantity, e.g. $2\frac{1}{2}$ litres, of air to pass through the meter, it will be found that if the barometric pressure corresponding to this mixture of atmospheric air and nitrogen is called P_1, then

$$P_1 = P \times \frac{T_n}{T_n + T_a}.$$

The altitude corresponding to a given barometric pressure can then be read off in metres or feet on any of the existing tables.

Example.—If it takes 18·2 seconds for 2·5 litres of nitrogen to pass through the nitrogen-meter, and 10·1 seconds for 2·5 litres of air to pass through the air-meter, then $T_n = 18{\cdot}2$ and $T_a = 10{\cdot}1$; and if the barometric pressure at the time of the experiment is 745 mm., we have an oxygen partial pressure corresponding to an atmospheric pressure of

$$P_1 = 745 \times \frac{18{\cdot}2}{18{\cdot}2 + 10{\cdot}1} = 463.$$

Therefore, the barometric pressure is equal to 463 mm., which corresponds to an altitude of 3,960 metres, or about 13,000 feet.

The advantages of the method are:

(1) That the apparatus is readily put together and can be used in any place, either indoors or in the open.

(2) It does away with all interfering influences of reduced pressure on the internal ear or other organs, and thus allows the pure study of the effects of decreased oxygen partial pressure.

(3) It is possible to investigate not only the changes in reaction time by means of specially adapted instruments, but also to conduct practical experiments, as, for instance, to determine the readiness with which a pilot or observer can orientate himself, aim, fire a gun, &c.

(4) The person conducting the experiment is not himself incapacitated by the effects of 'oxygen want', nor does he have to breathe oxygen to obviate these effects, as would be the case if a depression chamber were used.

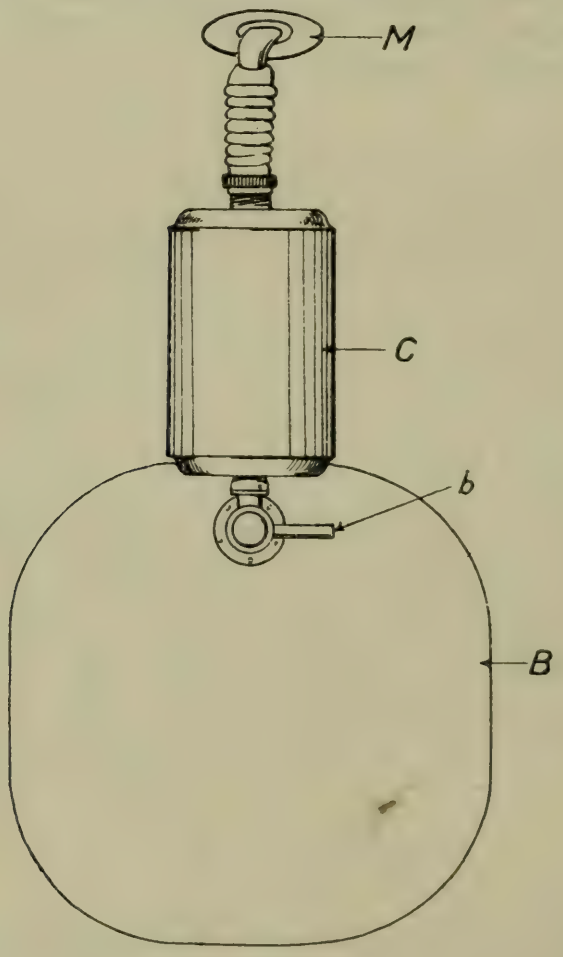
3.—THE BAG METHOD FOR THE INVESTIGATION OF AIR DISABILITIES OF AVIATORS

BY

Lieutenant-Colonel Martin Flack, C.B.E.

(*First circulated July* 1917. *Printed February* 1918. *Revised February* 1920.)

This apparatus is of service in affording a quick method of testing a pilot when more accurate means, such as the Dreyer apparatus



A. Method.

(previously described) or a rarefaction chamber, are not available. It consists of a rubber mouthpiece (M) fitting over a flat metal tube, which is joined by a flexible rubber tubing to an absorbent cartridge for carbon dioxide (C), which in turn is attached to a bag (B) of about 5 litres capacity. Just below the cartridge is a by-pass tube (b) through which the contents of the bag may be sampled.[1]

[1] The apparatus is manufactured by Messrs Siebe, Gorman & Co. 187 Westminster Bridge Road, S.W.

The subject is given the bag empty, is asked to adjust the mouthpiece comfortably in the mouth, and then to inspire deeply through the nose and blow several times into the bag to fill it. When the bag is just filled to distension a clip is placed on the subject's nose and he is asked to breathe quietly in and out of the bag until he feels he can go on no longer. At this point he expires as deeply as possible into the bag; the flexible tubing is clamped and a sample[1] of the contents taken by means of a vacuum tube from the by-pass, as much air as possible being taken from the cartridge by folding the bag about the main mass of air or closing the entrance hole to the bag with the hand.

This sample, which represents approximately the alveolar air after inspiration and expiration, is now analysed, and the percentage of oxygen in the bag determined. The time of the experiment is taken, also the time at which the breathing deepens appreciably, and any other symptoms such as blueness of lips, circumoral pallor, sweating, &c., are noted. After the experiment a brief account is taken of the subject's experience. From his statements and symptoms observed an opinion can be formed as to whether the subject suffers from 'oxygen want'.

B. Results.

The experiments with normal pilots show that in general the normal pilot will breathe the bag from 3½ to 4 minutes or more, and until the percentage of oxygen reaches 8 per cent. or under. It is interesting to note, however, that some of the pilots just back from hard work overseas do not reach this level. Whether this be due to lack of resolution or to accumulated symptoms of 'oxygen want' it is impossible to say, but the latter view is suggestive.

The chief value of the bag is in the confirmation of symptoms of 'oxygen want' said to be experienced by an aviator. Any one who really suffers from rarefaction of the air quickly develops a hyperpnoea, and usually has to give up breathing inside 2 minutes. When asked to describe the sensations experienced he usually volunteers the remark that they are the same as he gets in the air. Pilots who have fainted in the air have fainted momentarily with the bag experiment. The percentage of oxygen in the air of the bag generally gives an estimate of the height to which the aviator can go without unpleasant sensations. This, however, is not always the case, as a resolute man will push on beyond the stage at which the unpleasant symptoms begin to develop. The calculation is made as follows:

Taking 16 per cent. of oxygen as the content of the alveolar air on the full vital capacity at 760 mm. Hg (average of many estimations), then by simple proportion the number of millimetres of Hg corresponding to the percentage of oxygen can be calculated. Thus, supposing the bag content is 11·5 per cent., then

$$\frac{760 \times 11{\cdot}5}{16} = 546 \text{ mm. Hg.} = 9{,}000 \text{ ft. (approx.).}$$

[1] The carbon dioxide in this sample must not exceed 0·2 per cent., otherwise the result of the experiment is unreliable. The presence of unabsorbed carbon dioxide increases the power of the subject to continue the experiment, hence the calculated height will become too high.

TABLE GIVING RELATIONSHIP BETWEEN OXYGEN CONTENT OF BAG, BAROMETRIC PRESSURE AND ALTITUDE.

Percentage of Oxygen in Bag.	*Barometric Pressure* (15° C.) *in mm. Hg.*	*Approximate Altitude in feet.*
15	713	2,000
14	665	3,800
13	617	5,700
12	570	7,900
11	522	10,500
10	475	13,000
9	427	15,800
8	380	19,000
7	332	22,600
6	285	27,000
5	237	over 31,000

This has been confirmed several times by the use of the experimental rarefaction chamber. Lt. C., who breathed the bag until the oxygen content was = 17,500, staggered about and felt ill in the chamber in an atmosphere corresponding to 18,000 feet. 2/Lt. S , who with the bag felt symptoms, with a percentage corresponding to 9,000 feet, felt the same symptoms in the air badly at 9,500 ; generally slightly about 7,000 feet ; and in the experimental chamber at 10,000 feet.

In the following table is given the calculated altitude, by the bag method, to which the subject can fly before symptoms develop and the actual altitude at which symptoms have developed.[1]

No. of subject.	*Calculation height.*	*Height at which symptoms develop.*
1	Just over 4,000 ..	4,000.
2	6,000	4,000.
3	8,800	Max. 10,000 ; fainted twice above 8,000.
4	4,000	3–4,000.
5	Just above 8,000 ..	Max. 11,000 ; uncomfortable about 5,000.
6	7,000	4,000 (after long flight).
7	9,000	Max. 9,500 ; queer above 7,000. Confirmed in chamber at 10,000.
8	4,400	Pains in head, 3–4,000.
9	Just below 6,000 ..	About 6,000.
10	Nearly 17,000 ..	Headache after long time, 7–10,000.
11	6,000	Begins to feel suffocated at 7,000. Fainted since crash at 8,000 and 10,000.
12	16,000	Difficulty at 5,000, only four hours flying.
14	17,500	Vomiting twice after crash. Confirmed in chamber as 18,000.
16	3,000	Fainted three times below 5,000.
17	4,000	Above 3,000.
18	4,000	Dizzy and sick above 4,000.
23	4,500	Giddy and faint about 5,000.
25	5,500	Dizzy and headache, 5–6,000.
29	5,000	Pressure in head ; blurring of vision ; 5–6,000.
32	4,500	Giddy, 5,000.
34	10,000	Constant headache about 8,000.

It will be seen that in many cases the agreement is striking, but, as already mentioned in some cases, allowance has to be made for the fact that the subject pushes beyond the beginning of the unpleasant stage, e.g. Nos. 10 and 12.

[1] Many further cases have been examined since this table was compiled

The results are, however, sufficiently near, since the chief object of the bag experiment is to confirm the pilot's statement, and future flying will be confined to several thousand feet below that at which symptoms develop.

The bag is also of service in the diagnosis of the neurasthenic or the man suffering from lack of resolution. Such a person gives up the bag without exhibiting any marked symptoms, and does not generally describe any sensations to be attributed to want of oxygen. The neurasthenic usually ventures some such remark as 'beastly' or 'horrid'. One such could only breathe the bag for 38 seconds. On the other hand, some subjects unsuspectingly attain a diminution of oxygen far in excess of the utmost height to which they say they can ascend, as, for example, a man who always suffered anxiety at 5,000 feet reached a percentage of oxygen corresponding to 18,000 feet without much discomfort.

C. Conclusions.

(1) The bag method described is of service in confirming statements made by aviators as to disabilities presumably due to lack of oxygen.

(2) After analysis of the oxygen content of the bag, by simple calculation an estimate in most cases can be arrived at of the approximate height at which such symptoms should develop.

4.—A STUDY OF THE REACTION OF PILOTS AND OBSERVERS TO DIMINISHED OXYGEN-PRESSURE

BY

The late C. D. H. Corbett, M.D., M.R.C.P., late Lt.-Col., R.A.F., and H. C. Bazett, O.B.E., M.C., M.B., F.R.C.S., late Capt., R.A.F.

(*Printed November* 14, 1918. *Revised, with appendix, February* 1920.)

The investigations with which this paper deals have been carried out at Nos. 14 and 24 General Hospitals, B.E.F., as part of an examination of a large number of flying officers. It frequently happens that a flying officer complains of a group of symptoms of such a character as to suggest that they are caused wholly or partly by the diminution of oxygen-pressure which exists at high altitudes. Although in some cases the symptoms complained of in the air are capable of confirmation by evidence, medical and otherwise, obtained after landing, in other cases we have to depend solely on the individual statement of what happened many thousands of feet above the ground. Our observations have a practical value, a point on which we would like to insist. Their main object is in fact to determine whether or not the symptoms of which the patient complains are the result of oxygen-want. If this is found to be the case, it has further to be determined whether the individual is fit to continue the particular type of flying on which he has been employed, and in certain cases whether he is fit to continue flying of any description. In this connexion it must be pointed out that on active service there is no 'graduated flying'; and the pilot who is physically incapable of doing his full share of work merely increases the work of his brother officers.

In the course of our work we have had opportunities for studying the changes induced in the cardio-vascular and respiratory systems by exposure to air deficient in oxygen. We propose to describe these changes in some detail, but it is not our intention to enter into an academic discussion either of their physiological significance or causation. Our chief concern is to describe accurately such facts as we have observed and to emphasize those which appear to us of practical importance.

We should like to take this opportunity of drawing attention not only to the remarkable variation among individuals in their capacity to withstand lack of oxygen, but also to the varying character of the disturbances evoked by oxygen-want. Of these disturbances, dulling of perception is certainly the most important and probably the most common, while it is interesting to note that only

a small number of the patients examined gave a history of fainting in the air. It would also appear probable that the bodily changes by which the organism adapts itself to oxygen-deficiency are not of a standard pattern but are capable of a certain amount of individual variety.

Before proceeding to describe the methods employed and the results observed we propose to say a few words on the broad question of flying at high altitudes. In the first place, one cannot but be struck by the complete immunity from any discomfort which some individuals enjoy during arduous and protracted terms of high flying, and by the comparative immunity enjoyed by the vast majority of scout pilots. The conclusion is forced upon us that the normal healthy individual, especially if by virtue of his previous mode of life he has learnt to make full use of his lungs, can endure the strain of flying up to 18,000 feet for a considerable time without loss of efficiency. The more immediate explanation of this must be sought in the comparative immobility of the flying officer and his machine, and in the stimulating effect of the cold air on the face, the trunk and limbs being protected from cooling by suitable clothing.[1] The more remote explanation of this immunity is, however, to be found in the physical fitness of the individual, and in view of this we would strongly urge that the care of the body should form an essential feature of the training of cadets. We have seen numerous pilots who appear hardly to have heard of physical fitness and have never learnt to take any sort of pride in their bodily health.

Secondly, we wish to point out that out of a thousand flying officers who have passed through our hands no case has come to our notice of any organic changes in the respiratory or circulatory systems which could possibly be attributed to the effects of flying. It is possible, however, that long-lasting alterations in the mechanism which regulate respiration may be produced by a prolonged spell of flying at great altitude.

Thirdly, we do not suggest that the methods employed are necessary for routine use in the examination of candidates for the air service. The respiratory tests (1) described by Lieutenant-Colonel Flack (Report No. 7) are of great assistance to medical examiners in determining the physical fitness of an individual, and therefore his physical fitness for flying. Although it is true that an individual who is well below normal on the manometer test is useless for high altitude flying, we have found some who, while quite good at this test, are also incapable of enduring want of oxygen. Further, it is impossible to assess the degree of respiratory fatigue in tired pilots by this means unless some record has been kept of their normal performance.

Fourthly, it is evident that many officers proceed overseas with an unjustifiable dread of the effects of high altitudes, and it is not surprising that on their first flight to 15,000 feet or over the hyperpnoea of which they become conscious should alarm them and lead them to imagine that they are unfitted for such work. It would therefore appear of great advantage if pilots, and to an even greater

[1] Several officers when being tested by Dreyer's method have expressed a desire to 'put their heads over the side'

extent observers, who will be expected to fly at great altitudes overseas, could be sent up at least once in England to 15,000 feet, and kept at that altitude for at least 30 minutes, if only to be reassured and to ' see what it is like up there '.

In the earlier part of our work considerable use was made of Flack's bag, (2) and this method proved of great value if the changes in pulse-rate and respiration were observed. But although this test picks out many men who are incapable of flying at high altitudes, the results are often not conclusive, since an individual may be able to withstand these adverse conditions for a few minutes, while he is quite incapable of enduring oxygen-want for any length of time. The results obtained by this method will be summarized later in this report.

The exhaustion-chamber method has not been employed, partly on account of the expense and labour involved, but mainly owing to the introduction of Dreyer's method (3), which frees the examiner from the heavy handicap of being exposed himself to a rarefied atmosphere.

As compared with the bag test the Dreyer method, while certainly somewhat elaborate, reproduces much more exactly the conditions to which airmen are exposed. We have come to use it as a routine, and only use the bag when pressed for time. A few cases have been examined by both methods for the sake of comparison.

Up to the present time fifty cases have been examined by the Dreyer method, and of these a very large number were officers who had been overseas a very short time and were considered by their Squadron Commanders unsuited for high altitude work. In the majority of these cases the opinion of the Squadron Commander has been confirmed, but happily not in all.

A. Testing with Flack's Bag.

The method has already been fully described elsewhere. Some seventy cases have been tested in this way, some of them more than once, and one of us (D. C.) has experimented several times on himself. In most of the cases the pulse-rate throughout was taken by hand, and in a few instances the changes in respiration were recorded on a revolving drum, a pear-shaped bag, 6 inches long, with an internal spring, being strapped just below the xiphisternum and recording to a tambour. With the kind assistance of Lieutenant-Colonel Dreyer a careful investigation was conducted in some cases as to whether blood-concentration occurred.

It is necessary to emphasize one precaution that must be taken to ensure uniformity of results. If the caustic soda cartridge is not absorbing properly, varying proportions of CO_2 will remain which will entirely upset one's conclusions.

A proportion of CO_2 exceeding 0·3 per cent. assists the individual to breathe more deeply, so that he reaches a ' greater altitude ' on the apparatus, a lower percentage of oxygen being found on gas analysis. If the proportion rises above this the results are not comparable and the experiment is valueless as a test. There is no means of telling beforehand whether the cartridge is working properly;

if found to be defective the experiment was always repeated later with a new one.

The results obtained may be summarized as follows, the respiratory response being considered separately.

In addition, a table (Table A) is included, giving some instances of each class of case, together with some charts of the changes observed (Charts 1 and 2).

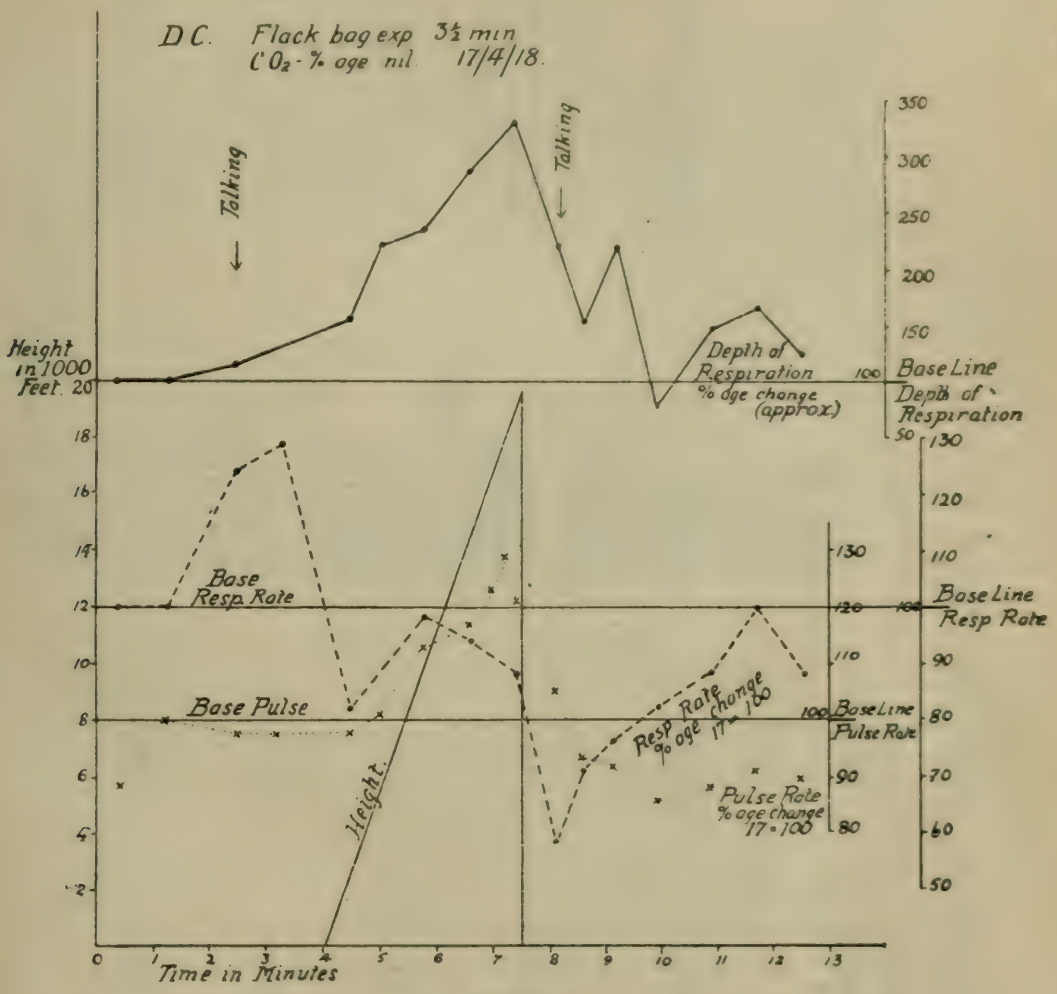


CHART 1

A. *The Normal Response.* A person of good physique and capable of withstanding altitude will persist for from 3 to 3½ minutes and an analysis of the air remaining usually gives 6 to 7 per cent. O_2, corresponding to 20,000 to 25,000 feet. That is apparently the average rate of consumption of O_2 in good types.

The pulse-rate should show a steady rise from the start. At the end it should reach about 100 per minute, and should not exceed 120. When the bag is removed it should at once fall and resume its normal rate in about 5 minutes.

B. *Abnormal Responses.* 1. Failure may occur as the result of cardio-vascular collapse. This type may faint and suffer subsequently from dizziness and headache. The changes in pulse-rate vary according to the type of case.

TABLE A. RESULTS WITH FLACK'S BAG

Type.	*Time.*	*Altitude.*	*% CO_2 in Sample.*	*Pulse Rate.*	*Remarks.*
Normal. (1) 2/Lt. C. J. F.	3½ min.	25,000 ft.	Nil.	*At start* 68 1 min. 80 2 min. 96 3 min. 112 3½ min. 120	Scout pilot. Due for home establishment. A little stale.
(2) Capt. G. C. M.	3¼ min.	25,000 ft.	0·17 %	*At start* 64 1 min. 88 2 min. 88 3 min. 104 3¼ min. 108	Scout pilot. Due for home establishment. A little stale.
Abnormal. I. A (1) Lt. C. E. T.	2¼ min.	16,800 ft.	Nil.	*At start* 92 1 min. 120 2 min. 128 2¼ min. fainting.	Headache and dyspnoea above 10,000 ft. Observer on prob. 40 hours in France Boarded permanently unfit flying.
(2) 2/Lt. J. F.	2¼ min.	14,000 ft.	Nil.	*At start* 100 1 min. 128 2 min. 144 2¼ min. fainting.	Dizziness at 8,000 ft. and over. 35 hours in France. Boarded permanently unfit flying.
B (1) 2/Lt. W. C. T.	2½ min.	15,000 ft.	0·1 %	*At start* 80 1 min. 100 2 min. 112 2½ min. 112 fainting.	Dyspnoea at 6,000 ft. and over. No flying in France. Giddiness on exertion. C.V.D. Boarded permanently unfit flying.
(2) Lt. R. L. R.	2½ min.	15,000 ft.	0·1 %	*At start* 56 1 min. 72 2 min. 80 2¼ min. 96 2½ min. 88 fainting.	Loss of sense of equilibrium at low altitudes. Boarded permanently unfit flying.

C (1) Lt. R. H. H.	1¾ min.	10,000 ft.	0·1 %	*At start* 114 1 min. 100 1½ min. 100 1¾ min. fainting. 4 min. later P. = 104.	180 hours on scouts. Very exhausted. D.A.H. type of pulse.
(2) Capt. G. C. W.	2¾ min.	30,000 ft.	0·6 %	*At start* 80 ¼ min. 100 1¼ min. 92 1¾ min. 80 2¼ min. 68 2¾ min 60 fainting. 15 sec. later 96 5 min. 92 6 min. 80	Fainted at 4,000 ft. when on patrol Rapid oxygen consumption probably due to CO_2 excess.
Abnormal. II. (1)	2½ min.	22,600 ft.	Nil.	*At start* 80 1¾ min. 100 2 min. 156 syncope.	Giddiness in air 10 hours in France.
(2)	2¼ min.	20,000 ft.	Nil.	No record. Gave up. Felt heart beating.	Giddiness in air. 80 hours in France.

(*a*) In cases who are physically incapable of standing altitude and may have done little flying the rate rises rapidly and may become uncountable. Syncope may occur early.

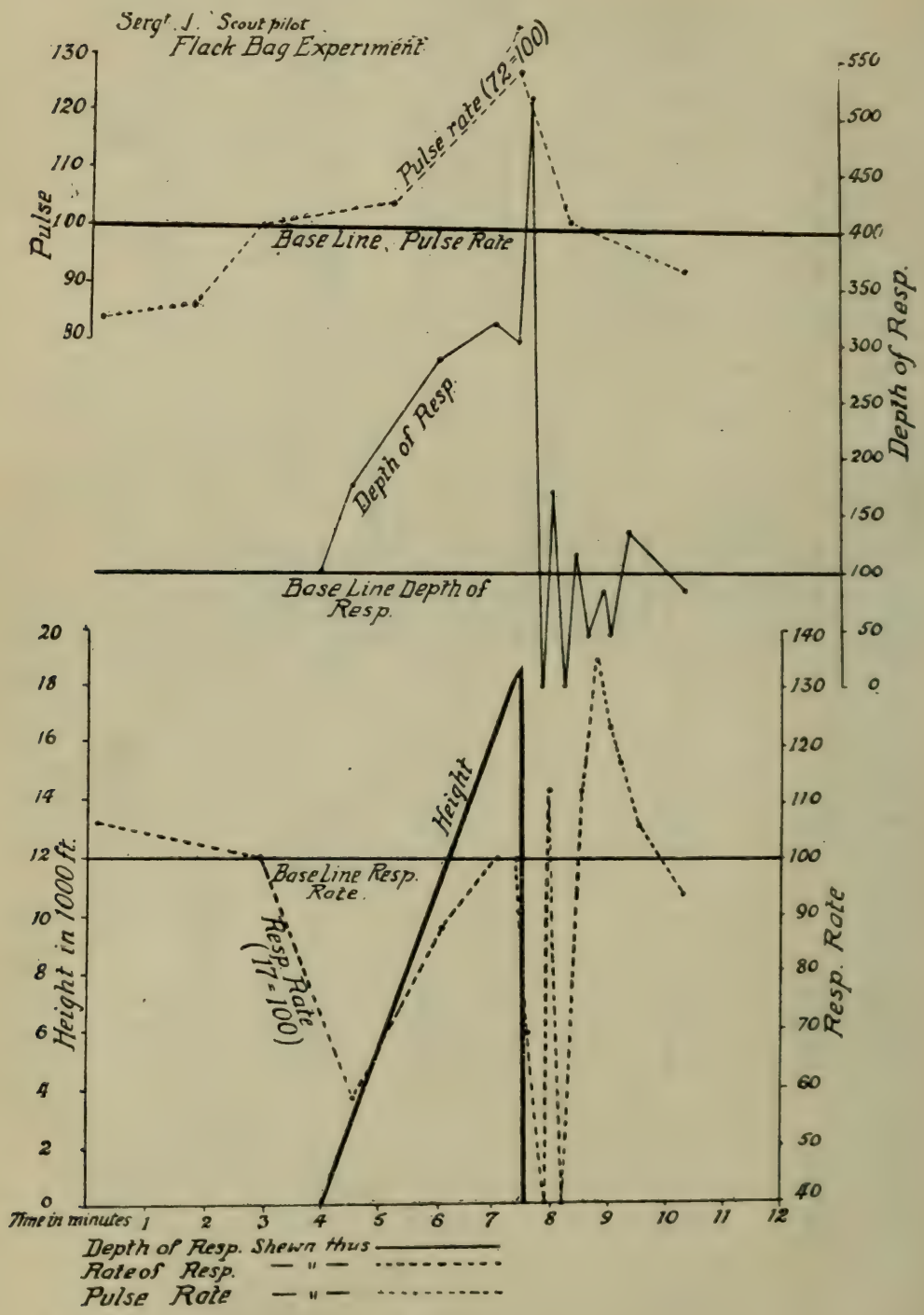


CHART 2

(*b*) In others syncope may also occur at a comparatively low altitude, but without an excessive rise of pulse-rate.

(*c*) In cases of oxygen-want, due to exhaustion, who have done a great deal of flying with perhaps a history of fainting in the air, the pulse-rate may rise during the first half-minute and then remain

stationary or gradually fall below its rate at the start. As soon as the bag is removed the rate rises.

2. In another type the oxygen consumption is abnormally rapid, leading to failure in under 3 minutes at an 'altitude' reached by a good type in 3 to 3½ minutes.

(a) The Respiratory Response.

Only in a few cases was the respiration recorded. In every instance the depth at first increased, and the rate remained slightly raised or stationary, although in good types it was diminished. In these latter the depth was maintained, while a response was considered to be bad when the respiration became more shallow towards the end and when periodic breathing occurred when the bag was removed.

(b) Blood Concentration.

This was observed in some cases, but it was a very transitory phenomenon. It was found within 2 minutes of the removal of the bag, and had disappeared 4 or 5 minutes later. The maximum concentration seen was under 5 per cent. No conclusions could be drawn from the results obtained as to its value from the point of view of diagnosis.

Appended are some details of bag experiments where the respiration was simultaneously recorded.

A. Normal response.

D. C. has never flown at high altitudes.

Height reached 20,000 feet in 3½ minutes.

Pulse rose from 77 to 100 per minute at 3¼ minutes, falling to 68 after the bag was removed.

Respiration.

(*a*) Rate fell steadily throughout the experiment.

(*b*) Depth steadily rose.

There was a compensatory rise of both rate and depth after the experiment, with very little tendency to periodicity. 20,000 feet is the usual height reached by D. C. in 3½ minutes (Chart 1).

B. Abnormal.

1. *A. D. S.* History of oxygen-want at 18,000 feet, evidence of cardio-vascular debility, pulse easily raised by exercise. The case is interesting in that it shows the effect of the presence of 1 per cent. CO_2 in the bag in greatly increasing the 'altitude' reached in a given time. The general condition of the patient was about the same at the end of the two experiments.

(*a*) With CO_2 1 per cent.

Height reached 26,000 feet in 3½ minutes.

Pulse rose from 89 to 133 at 3 minutes.

Respiration an increase both in depth and rate.

(*b*) With CO_2 nil.

Height 14,000 feet in 3 minutes.

Pulse rose from 96 to 115 at 2½ minutes, and then fell a little, and was probably falling towards the end of the experiment.

Respiration not recorded.

Of the two experiments the second alone gave any accurate

information as to the capacity of the patient to withstand oxygen-want, which was really rather poor.

2. *Sgt. J.* History of breathlessness at 12,000 feet, and occasional attacks of breathlessness night and morning.

Height 18,400 feet in 3½ minutes.

Pulse rose from 80 to 97 only, a subnormal response.

Respiration.

(*a*) Rate fell at first, rising to normal.

(*b*) Depth was very rapidly increased with marked hyperpnoea just before the bag was removed.

At the end of the experiment there was very definite periodic breathing, the rate showing 100 per cent. variations, while the depth showed only slight corresponding changes.

This patient was evidently of the type liable to periodic breathing at altitudes, which we have been able to show in our later work is often associated with a subnormal cardio-vascular response (Chart 2).

3. *Lt. P. E. H.* Fainted at 14,000 feet, recovered at 4,000 feet.

This case comes under the heading 1 (*a*) above of abnormal responses. He was subsequently tested on the Dreyer apparatus, and a chart of this experiment (Chart No. 11) is given later.

Height 17,000 feet in 2 minutes.

Pulse rose from 92 to 156, and was still 104 2½ minutes after the bag was removed.

Respiration.

(*a*) Rate rose slightly.

(*b*) Depth was very considerably increased.

In this instance syncope apparently occurred from cardio-vascular failure only, the respiratory response being well marked, though somewhat excessive. On the other hand, on referring to the Chart No. 11, illustrating the Dreyer experiment, it will be seen there that the failure was essentially respiratory in origin.

Tests by Breathing through a Long Tube.

A certain number of experiments (15) have been made in which the patient has breathed through a long tube, so that his supply of fresh air has been in this way much diminished, while the carbon dioxide has been absorbed by caustic soda. The patient wears a nose-clip, and breathes through a short flexible tube connected with a tin containing coke, through which runs a constant stream of 20 per cent. caustic soda. The lower end of the tin has an opening connected with large-bore rubber tubing, the length of which can be altered so that the supply of fresh air can be increased or decreased at will. The tubing used was of ¾ in. diameter, and lengths of tubing varying from 42 cm. to 185 cm. were employed. The dead space was in this way increased by a volume of 1,160 c.c. below the caustic soda and 70 c.c. above it, a total of 1,230 c.c. with the greatest length of tubing and with a length of 142 cm. (the length most commonly employed) 1,120 c.c. From the short flexible tube above the caustic soda receptacle a piece of pressure tubing is led out, allowing samples of alveolar air to be taken at any time during the experiment.

This method was tried by one of us (H. C. B.) in an attempt to find

a portable apparatus for testing for altitude, but it has not proved satisfactory for this purpose. The apparatus is practically the same as that employed by Douglas and Haldane in their investigation of Cheyne-Stokes (4) breathing. In any patient with a given length of tube a very constant ' altitude ' will be maintained and the blood-pressure and pulse-rate changes correspond very closely with those seen under the conditions of a Dreyer test, but it seems to be impossible to decide how long a tube any patient will require to maintain him at a reasonable ' height ', and the degree of oxygen-want to which he has been subjected can only be decided by alveolar air analysis. While in one subject an increase of 40 cm. in the length of the tube would make a difference of 5,000 feet in altitude, and the patient would reach 23,000 feet with a length of 142 cm., and another would only reach 12,000 feet with a length of 185 cm. No relationship could be found between the ' altitude ' reached and either the vital capacity or normal tidal air of the individual, but the results seemed to depend on whether he responded by breathing deeply. The ' altitude ' reached has been calculated from the percentage of oxygen found in the alveolar air, 14 per cent. being taken as the normal. Owing to the difficulty of deciding what length of tube should be employed in any case, the method has been entirely given up as a practical test. For though the results obtained, if the respiratory and cardio-vascular changes are recorded, are of greater value than those obtained by the Flack's bag, this method is rather more troublesome to use, and the test must be continued for at least 30 instead of 3 or 4 minutes. That the effect produced corresponds closely with that obtained by Dreyer's method has been demonstrated by subjecting four people to both tests. A remarkable similarity was noticeable in the reactions observed, the only difference being a slightly greater rise of systolic blood-pressure in the tube experiments. This was probably due to the amount of work the subject had to do in order to obtain his air. Charts 6, 10, and 12 show the results obtained when the same patients were tested both by this and by Dreyer's method.

The tube method is therefore only described here, owing to the light it throws on the changes in the alveolar air, resulting from a degree of oxygen-want similar to that used in a Dreyer experiment. It was found that the CO_2 percentage remained constant in most cases at ' heights ' below 15,000 feet. Where the oxygen percentage in the alveolar air had been reduced to about 50 per cent. of normal (i.e. 18,000 feet) the CO_2 percentage was reduced slightly, an average of 6·1 per cent. being found at the commencement and 5·64 per cent. at the end of the experiment (about 30 minutes).

In marked contrast is one case of the excessive respiratory reaction type (see Chart 10 and Table C, 36). In this patient the respirations became excessively deep and rapid, so that the altitude reached was less than 9,000 feet and was quickly lowered still farther, while the CO_2 percentage starting at 6·1 per cent. was reduced to 4·5 per cent. Although no deductions can be drawn from a single experiment this result is suggestive, considering the extraordinary excessive respiratory response, and especially as no such change in the alveolar air was ever observed in the more normal subjects tested by this method.

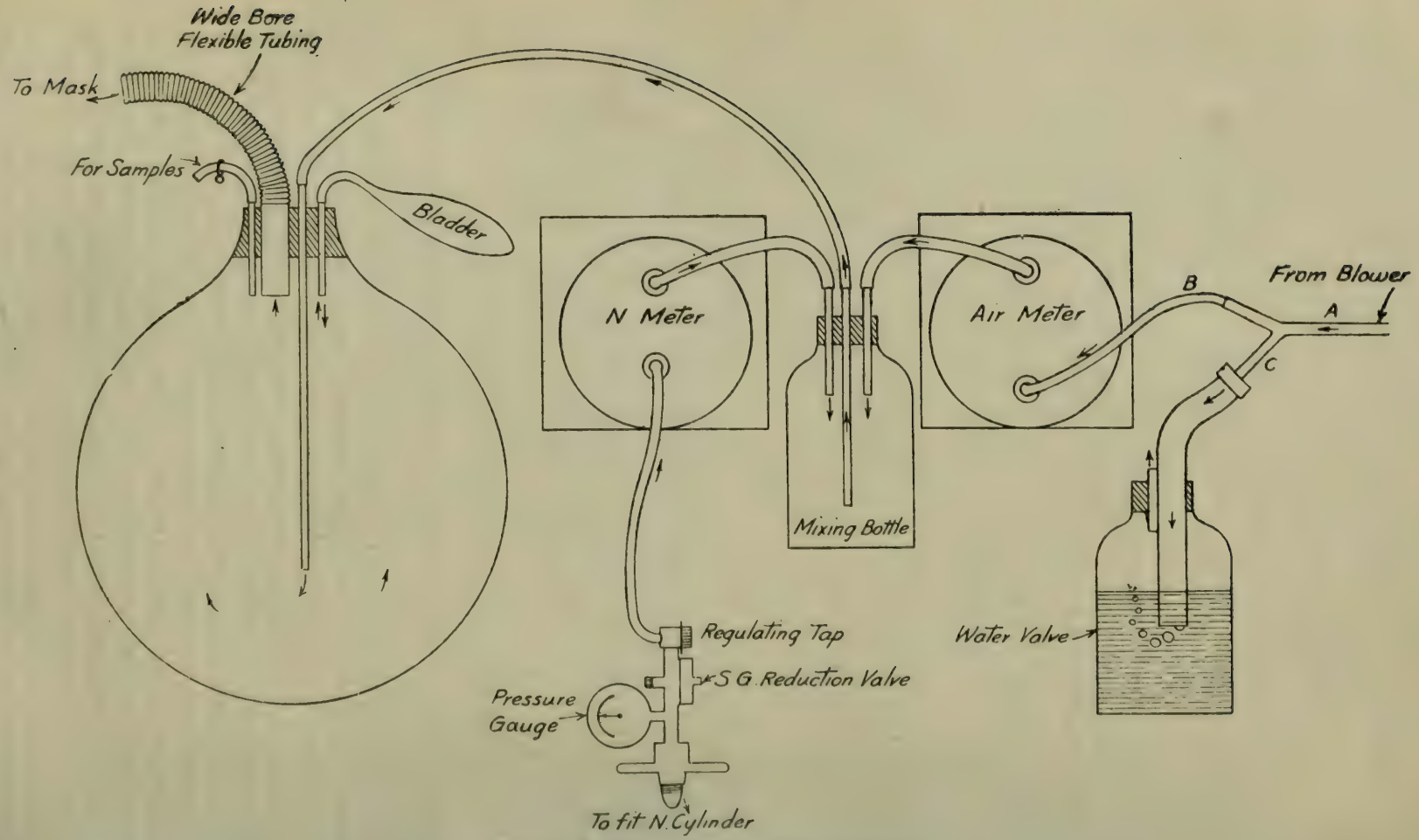


FIGURE I. Diagram of Dreyer Apparatus.

B. Dreyer's Method.

(*a*) *Apparatus.*

It was found necessary to make certain modifications in the apparatus as originally figured by Colonel Dreyer. In the first place a large mixing bottle was used instead of the T-piece, in order that the air and nitrogen might be well mixed before passing on to the large reservoir. In the place of a large bell-jar we used a glass sulphuric acid carboy of 40 litres capacity, as this can be easily obtained.

There was at first considerable trouble with the air-supply, the current available for the motor being very variable, so that one could not ensure a constant mixture.

The governing device illustrated was found to be very useful in getting over this difficulty, and consists in a simple water safety-valve (Fig. I).

(*b*) *Description of Water-Valve.*

The air from the blower is by a tube 'A' to a T-tube of quite ½ in. internal diameter. From one aperture of this a tube 'B' goes to the inlet of the air-meter, and from the other a tube 'C' is connected with a tube of ¾ in. bore, dipping into a large wide-mouthed bottle three-parts filled with water. Some device must be used to fix the tube at any desired depth without restricting the outflow of air from the neck of the bottle. When the motor is started its rate is so adjusted, and the tube is set at such a depth in the water, that the air just bubbles out from its distal end, and the air meter shows 2·5 litres in 7·5 seconds (this being the volume of air delivered to the patient before the nitrogen is turned on). If the motor happens to slow down slightly the resistance of the water will prevent any escape of air from the tube, or again, if its speed is increased somewhat the excess of air delivered will bubble away instead of going through the meter. When it is desired to reduce the amount of air delivered the tube is simply pulled up, allowing more air to escape while the speed of the motor is kept constant. In this way a very steady and accurate volume of air can be delivered.

For the delivery tube to the mask we used Douglas flexible tubing of 1 in. internal diameter, as a narrow tube should be avoided.

A small piece of pressure tubing was fitted into the mouth of the large reservoir in order that samples of the mixed gases might be taken for analysis. As a general rule this is not necessary and was only done occasionally as a control.

(*c*) *Method of Working.*

The motor is started and adjusted with the water-valve to deliver 20 litres of air per minute to the patient. It has been found that this supply has been ample in all cases tested to keep the patient comfortable even in case of hyperpnoea, and throughout the experiment the total volume of mixed gases delivered was kept constant at 20 litres. Before deciding to what altitude the patient should be taken, attention is paid to the history of the case. Three stages have been generally used, 12,000 feet, 15,000 feet, and 18,000-

19,000 feet. For these the meter times for one complete revolution (2·5 litres) are as follows :

		Air.	*Nitrogen.*
Stage 1.	12,000 feet	10·5 secs.	19·5 secs.
Stage 2.	15,000 feet	13 secs.	17 secs.
Stage 3.	18–19,000 feet	15 secs.	15 secs.

It might be as well here to requote Col. Dreyer's formulae for calculating the equivalent barometric pressure of the mixed gases :

$$P_1 = P \times \frac{Tn}{Tn+Ta}$$

Where P_1 = barometric pressure looked for.
P = barometric pressure at time of expt.
Tn = time for $2\frac{1}{2}$ litres of N to pass in seconds.
Ta = time for $2\frac{1}{2}$ litres of air to pass in seconds.

It has been found that pilots are better able to stand altitude in air where the barometer is much above normal, the converse being also true. Similarly, when the barometer is high patients are able to stand a lower percentage of oxygen when tested by this method.

The average barometric pressure at different altitudes can be seen according to the following table :

Feet	Mm. Hg.
8,000	570
10,000	530
12,000	494
14,000	460
16,000	428
18,000	398
20,000	370

The patient is usually taken up to 15,000 feet straight away, and it has been calculated that he will reach this height in between 5 to 10 minutes owing to the dead space of the apparatus. The observation of the patient is dealt with below. Care should be taken as far as possible to cut down the air and turn on the nitrogen without attracting his attention. If the reaction is unsatisfactory or if there is anything in his history to warrant it he is kept at this height, otherwise after a short time he is taken to the next stage, where he stays until the end of the experiment, or he may be brought down again to the lower level.

Early evidence of syncope will be afforded by a considerable fall of blood-pressure, preceded by a gradual rise of diastolic pressure. As soon as this or any other indication occurs for stopping the experiment the nitrogen is turned off and the supply of air increased to its original volume, the mask being kept on. In this way the after-reaction can be observed under the conditions of the experiment. As soon as the air is turned on, as described, the patient is almost immediately aware of his improved conditions, although, as before, the change from one level to another will take from 5 to 10 minutes.

In a few cases it was necessary to cut short the experiment by removing the mask before the nitrogen was turned off.

The sensations experienced by the patient correspond very closely with those produced in actual flying conditions, and many pilots have been able to estimate correctly the artificial altitude to which they were being exposed. A statement by one of them, an experienced flight commander, is appended :

' In my opinion the Dreyer apparatus for testing pilots for high flying is extraordinarily good.

' The sensations experienced at 20,000 feet in the air are exactly the same as those produced when tested by this apparatus on the ground at normal atmospheric pressure.

' During the test, when the amount of oxygen breathed was greatly reduced, I felt very cold in the feet and hands, symptoms which I had previously attributed to the intense cold experienced at great heights.

' The feeling in my head was exactly the same except that I did not feel as if my head were bursting, as is the case when one is flying at a great height for a long time.

' The mask does not restrict the breathing in any way.'

(*d*) *Blood-Volume Changes.*

Time has not allowed any thorough investigation of this subject, and the number of observations made has not been very great. Up to the present time no samples of blood have been taken from veins, but only from the capillary circulation, and it has been assumed that the blood-volume has varied inversely as the proportion of haemoglobin in the peripheral blood. Four drops of blood are taken from an ear or finger without squeezing into a paraffined watch-glass, and from this 0·05 c.c. is sucked up into a graduated pipette and added to 9·95 c.c. of normal saline in a test-tube, thus making a 1-in-200 suspension. The test-tube is gently rotated to allow mixing, and then no clotting should take place, if the saline has been made with clean distilled water. A number of samples are taken in this way, before, during, and after a Dreyer test. These blood-suspensions are allowed to settle and will keep a few hours at room temperature, but as soon as possible after the experiment, when all the blood-samples have been collected, the test-tubes are again gently shaken to mix and a few grains of saponin are added to each to cause haemolysis. The resulting haemoglobin solutions are compared with each other in the Duboscq colorimeter, artificial light in a dark room being used for this purpose. In this way even small changes can be detected and carefully measured. The original samples are taken before the patient is submitted to oxygen-want ; these should agree with one another and are reckoned as 100 per cent., while the others are all compared with them, the percentage change being estimated. Care has to be taken to obtain the blood under exactly similar conditions, without squeezing and in the same amount, and the time taken between bleeding and sucking up the blood in the pipette should be kept as constant as possible, as settling of the corpuscles quickly occurs and may cause errors. For this method, which has proved extremely accurate in use, we are again indebted to Col. Dreyer.

(*e*) *Cyanosis.*

Cyanosis develops in all patients at or about 'heights' of 18,000 feet, but it comes on slowly and is not seen as soon as that height is reached. No definite differences have been noticed in this respect between various individuals.

(*f*) *Physical Work.*

Physical work has been used while the patients were subjected to oxygen-want to increase the severity of the test. As far as possible this work has been made similar to that which might have to be done in the air. The most useful test has been the blowing up with a pressure pump of an imitation petrol tank, the patient being required to raise the pressure to four atmospheres in spite of a leak which is always tending to lower it. The effect of this on his respiration and pulse-rate has been noted. On other occasions the patient has been made to stand up.

(*g*) *Mental Work.*

The ability of the patient to perform mental work has been tested chiefly by arithmetic. A simple division sum has been given him when he has been receiving pure air wearing the mask, and this sum is repeated when he is at an 'altitude'. The time taken over the sum is recorded in each case, but is invariably longer when the patient is exposed to oxygen-want, in spite of the fact that he is repeating the sum for the second time. The patient is peculiarly liable to mental symptoms at altitudes, gross errors are made in the sum, and he is often quite incapable of finishing it.

(*h*) *Subjective Symptoms.*

The patient will usually complain of a number of symptoms such as headache, sleepiness, shortness of breath, praecordial pain, abdominal pain, dizziness or faintness. Only one case has shown any inclination to vomit. The symptoms are noted. If carried sufficiently far the sensations resemble very closely those of a man going under an anaesthetic, but it is quite unnecessary to carry the experiment to this length. On returning to full air the headache, if it has been present, usually continues for some hours or may even last a day or two. Of all symptoms headache and sleepiness are the most common.

C. Observation of the Patient.

Care must be taken to exclude excitement in the patient. The mask should be explained to him, and then he should be allowed to sit quietly in a chair with the mask on and a supply of pure air until the circulation and respiration have settled to their normal values for rest; 10 to 15 minutes is usually sufficient for this purpose. It is also an advantage to supply the patient with some literature to keep his attention occupied during the progress of the test, and incidentally this will help to distinguish those who quickly lose their power of mental concentration at an altitude. Good results may be obtained without any special recording apparatus, by observing the

general condition of the patient, but it is much better to keep a careful record of the respiration, pulse-rate, and blood-pressures, since by these means commencing failure may be recognized earlier, and so the duration of the tests may be shortened. It is inadvisable for many reasons to carry the experiment to the point where complete collapse occurs, and the mask should be at once removed if syncope is imminent. The methods that have been employed for the observation of the patient must therefore be described.

(*a*) *Respiration.*

The respiration has in many cases been recorded by means of a pear-shaped bag strapped over the xiphisternum and connected to a tambour. This method has already been mentioned in connexion with the results obtained with Flack's bag, and on the whole gives a record more or less proportional to the depth of respiration. Its disadvantage lies in the fact that if the respiration becomes very shallow and is costal rather than diaphragmatic, it may be scarcely recorded at all. None the less with most subjects the record is fairly accurate.

More recently the records have been obtained by connecting the expiratory tube of the mask with a delicate tambour. A rubber tube is slipped over the expiratory valve and the outlet is clamped and constricted sufficiently to create a slight positive pressure in the mask and glass reservoir. This should be just sufficient to cause the football bladder to remain half distended without being in any way tense. This degree of resistance to the outflow is insufficient to hamper the patient, and it prevents the mask being sucked forcibly against his face and so makes him more comfortable. In addition, by helping to keep the football bladder partly filled it increases the reserve of air available for the patient, should he want to take a rapid deep breath. The record is then obtained quite easily by letting in a small glass tube into this rubber cap, and connecting with a very delicate rubber tambour. During inspiration the expiratory valve is closed and the tambour registers atmospheric pressure ; while during expiration the pressure will vary according to the rate at which the air is expelled. A shallow quick expiration will produce a spike, a deep slow expiration will give a maintained raised plateau ; but if the area covered by the curve is measured and not merely the height reached, then the volume of the respiration may be roughly estimated from the curve. Naturally the recording pen must have as little friction as possible, therefore the rod carrying it should be light and flexible.

In practice the records obtained in this way have been found to be useful and reasonably accurate, and, by glancing at the record, an estimate of the respiratory reaction can usually be made. But where the results have been plotted on a curve, considerable labour has been involved. Periods of 15 to 30 seconds have been chosen at intervals of a few minutes at typical parts of the charts, and the rate has been determined by counting the respirations in this period. The average depth of the respirations has then been estimated by finding the average height and average width of the same curves, and then obtaining the product of the two. A more accurate result

might be obtained by taking the product of the height and breadth of each wave separately, but the former method is quicker and sufficiently accurate. If the wave is of the nature of a spike the breadth is taken as that of its midpoint. Finally, all the results have been expressed as a percentage of the respiration seen at the commencement when the patient was receiving pure air.

This method gives a real indication of the changes in the ventilation rate of the patient. This is borne out by the fact that during the preliminary period when the patient receives pure air, the ventilation rate (i. e. the respiration rate multiplied by the average depth) remains usually remarkably constant even when the respiration rate is showing considerable variation, and also by comparing the records of several individuals obtained in this way with the values for their tidal air, &c., obtained by making them breathe into a bag.

(*b*) *Pulse-rate.*

This has been taken by hand at frequent intervals, and care is taken to ensure as far as possible that the original pulse-rate is the normal for the patient at rest.

(*c*) *Blood-pressure.*

The observations in the blood-pressure changes have been made with the ordinary Riva Rocci apparatus with a mercury manometer, and the recurrence of the pulse has been determined by using a stethoscope over the brachial artery at the elbow, the diastolic pressure being measured at the same time. In arriving at the diastolic pressure the point at which the sounds begin to fade and become distant has been taken—as described by Oliver. With most patients this point is very definite and distinct, and any two experienced observers will get identical readings, but there are exceptional cases in whom the different phases are very indefinite. In these individuals the sound heard in the first period is usually hard and sharp, and the maximum loudness occurs very early ; as the pressure is allowed to fall the intensity of the sound gradually diminishes, and no definite point can be distinguished where the third phase changes into the fourth. Under these circumstances the reading of the diastolic pressure is uncertain. It is of some interest that it is not uncommon to find an individual who gives a definite diastolic reading at ground levels change so that the sounds become of this abnormal type when he is subjected to oxygen-want, while in other cases the sounds become more complicated and may disappear, to reappear again when the pressure is further lowered. Both these abnormal types have only been observed up to the present time in people who are unfit.

As a general rule we have found the pulse-pressure increased by a lowering of the diastolic pressure, in the reaction to oxygen-want. That the readings of diastolic pressure are correct in these cases is borne out by a more careful examination of the sounds. In a few cases the occurrence of the first sound, the pressure at which the sound is loudest, the point at which it changes from the third to the fourth phase, and that at which all sounds disappear, have

all been noted, and the changes observed as the patient was subjected to oxygen-want ; it was found that these sounds were all heard over a greatly increased range, but their relationship to each other

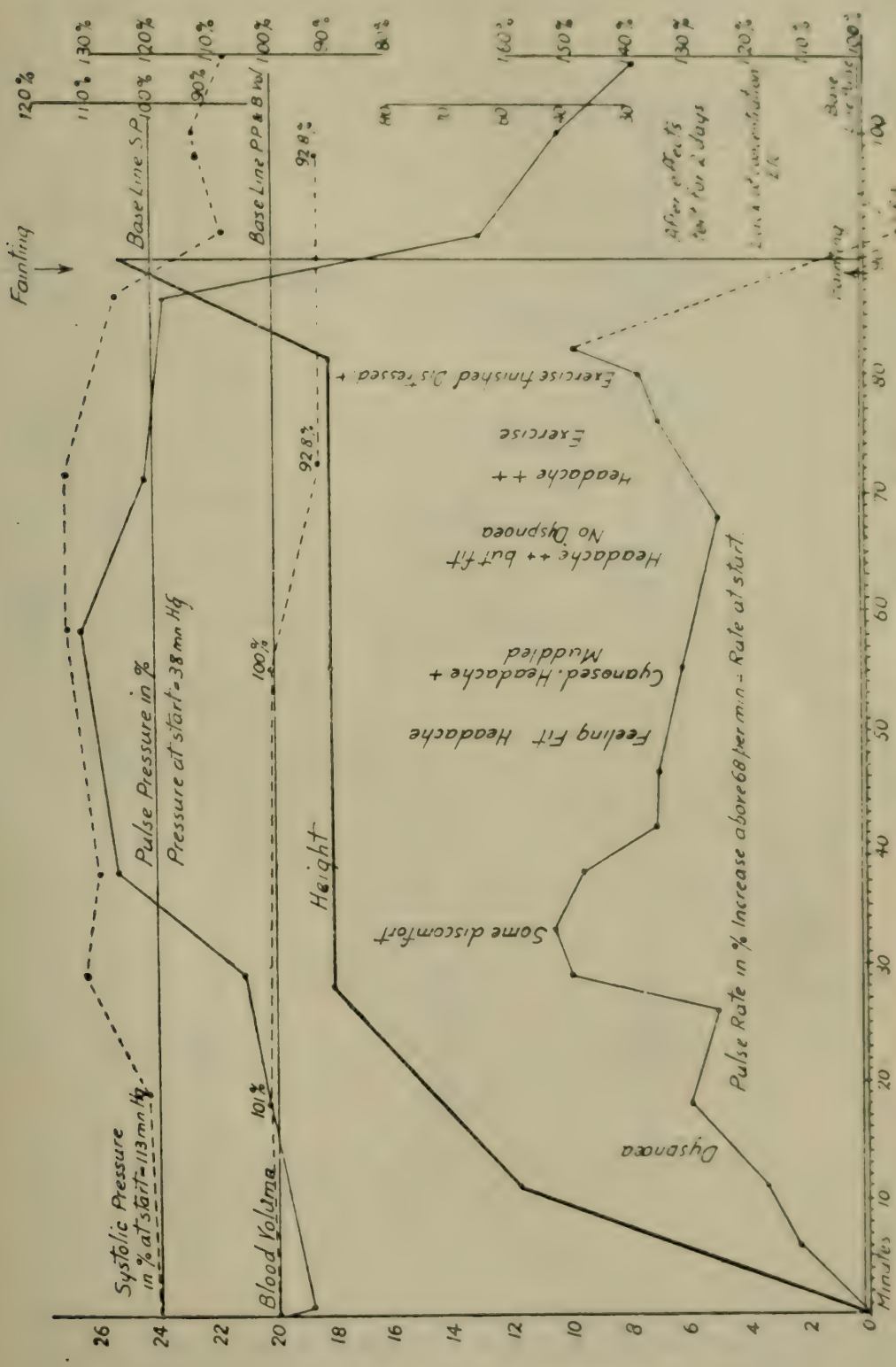


CHART 3. H. C. B. 24/2/18. (See Table B, 5.)

remained unaltered in ordinary normal individuals, and the diastolic pressure was lowered, whichever change in the sound was taken as the indication of the diastolic pressure.

D. The Reaction and its Interpretation.

The individuals tested may be classed roughly into two groups, those able to stand altitude well and those whose reaction is inadequate to enable them to fly at great heights for any length of

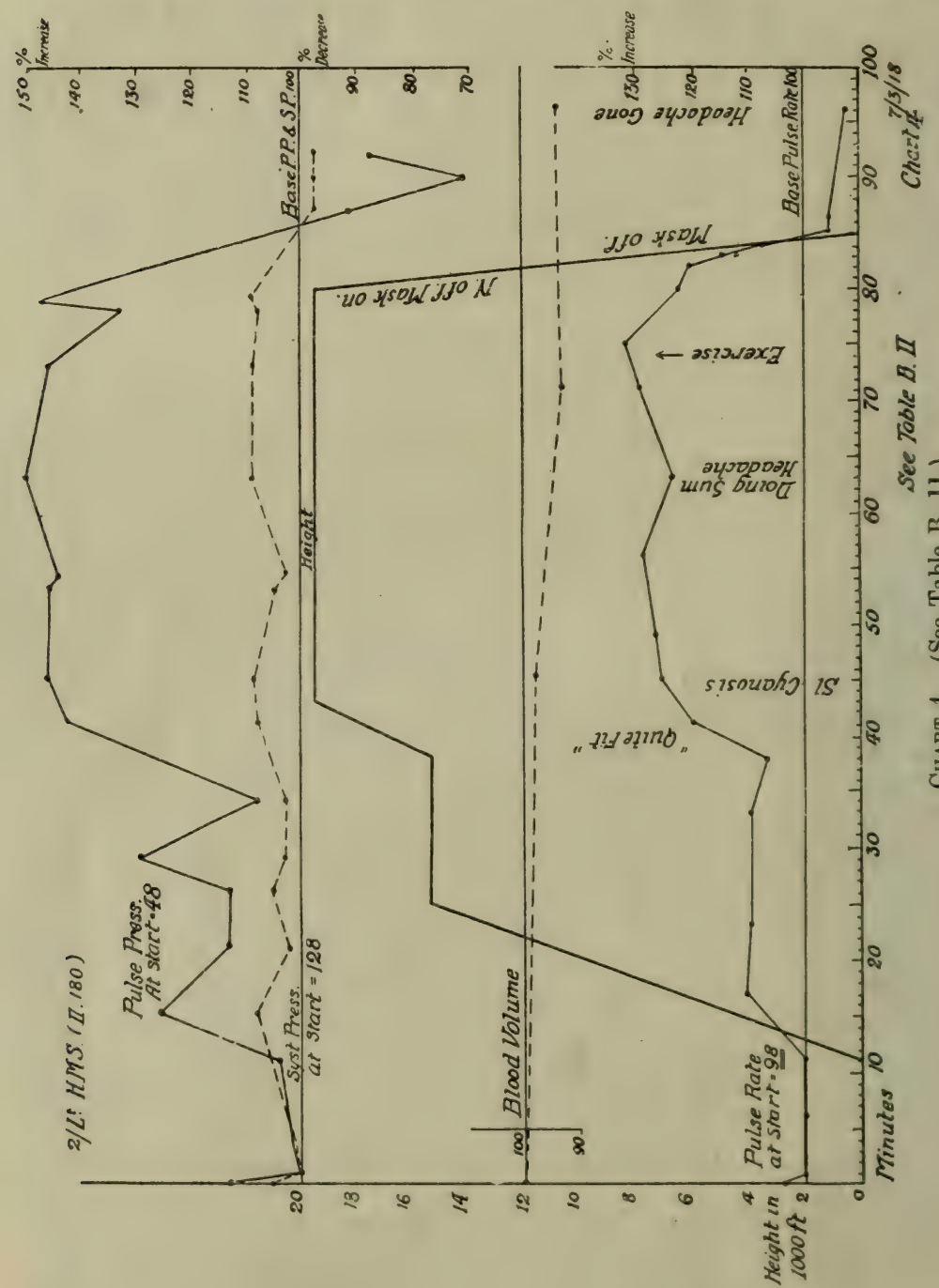


Chart 4 (See Table B, 11.)

time. In the good type there has been recognized a definite reaction both of the respiratory and cardio-vascular systems, although the relative importance of the changes seen in these two systems varies in different individuals. Similarly, the bad type may be subdivided into different groups according to the relative prominence of the changes observed.

(a) *The Good Type.*

The reactions observed in this type may be seen by reference to Table B, Charts 3, 4, 5, 6, 7, and 8, and Fig. II. Briefly, those in this group can withstand an altitude of 18,000 feet for an hour or more without undue distress. A few cases have complained of headache and others of slight sleepiness. The respiratory reaction was only carefully recorded in five cases. In these the depth was

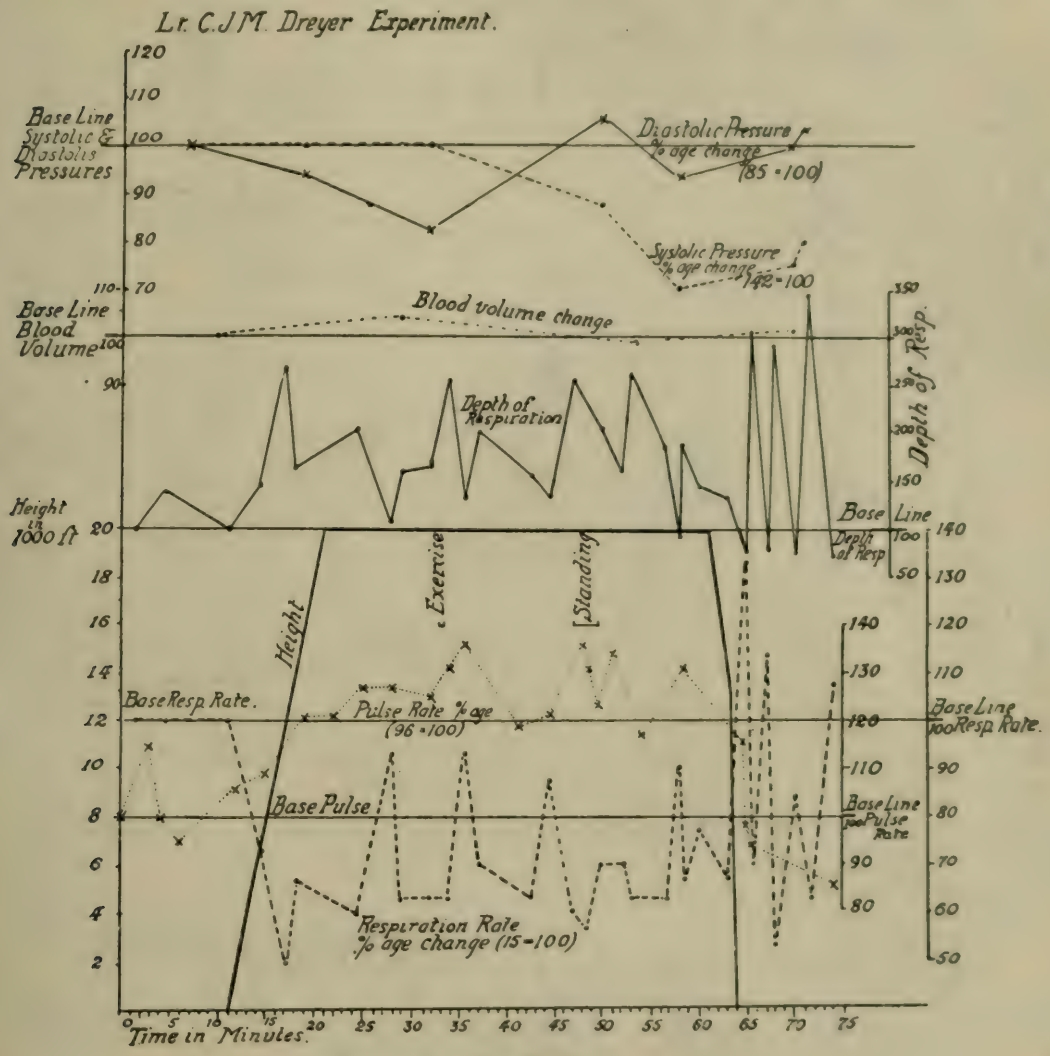


CHART 5. (See Table B, 20.)

increased, slight periodicity was common, and the rate was usually slowed. In one case (No. 44) the rate was also increased, but this officer had done little high flying, and it is doubtful whether he would stand much work at high altitudes (Chart 8). Officers of a good and intelligent type have often found that the dyspnoea experienced at high altitudes may be alleviated by educating themselves to breathe more deeply and slowly when distressed, and there can be no doubt that this is the normal good reaction.

The pulse-rate is increased about 30 per cent. above normal, but as adaptation takes place it tends to fall to a slightly lower level; this corresponds to the time at which blood concentration first makes

its appearance. In one case, Table B (42), the altitude reached was only 10,500 feet, with only a slight rise, but it is improbable that the normal individual increases his pulse-rate to any extent at this altitude. At the greater heights (18,000 feet), if the pulse-rate is not increased, unpleasant symptoms are inevitable. Parallel with the

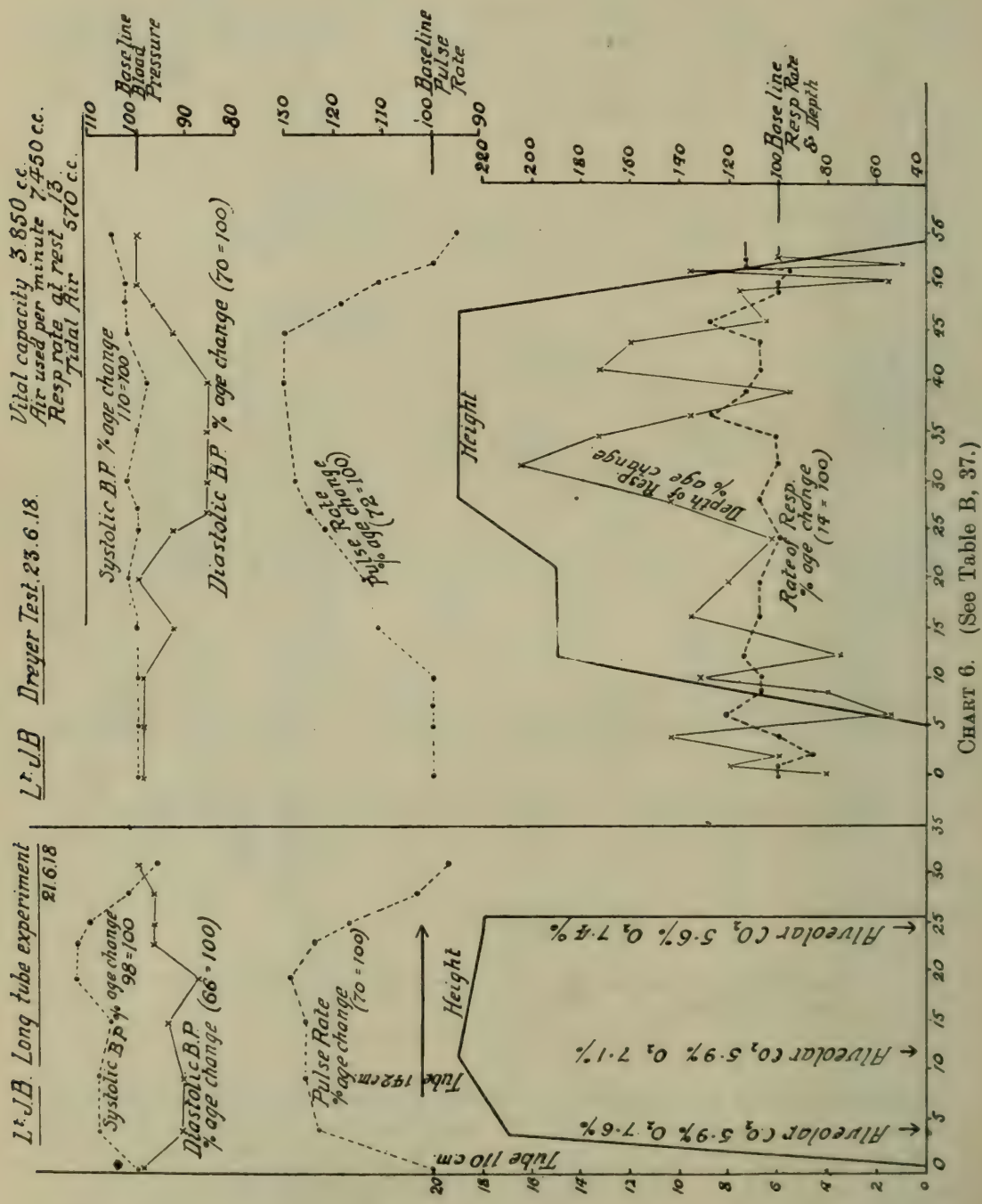


Chart 6. (See Table B, 37.)

rise in pulse-rate there is an increase in pulse-pressure usually brought about by a fall in the diastolic with only a slight rise in the systolic pressure. Where in this class a rise in pulse-pressure has been due mainly to an increased systolic pressure, mental or physical work has apparently been the cause. The diastolic pressure has never in the good type fallen below a level of 60 mm. Hg, and it is probable

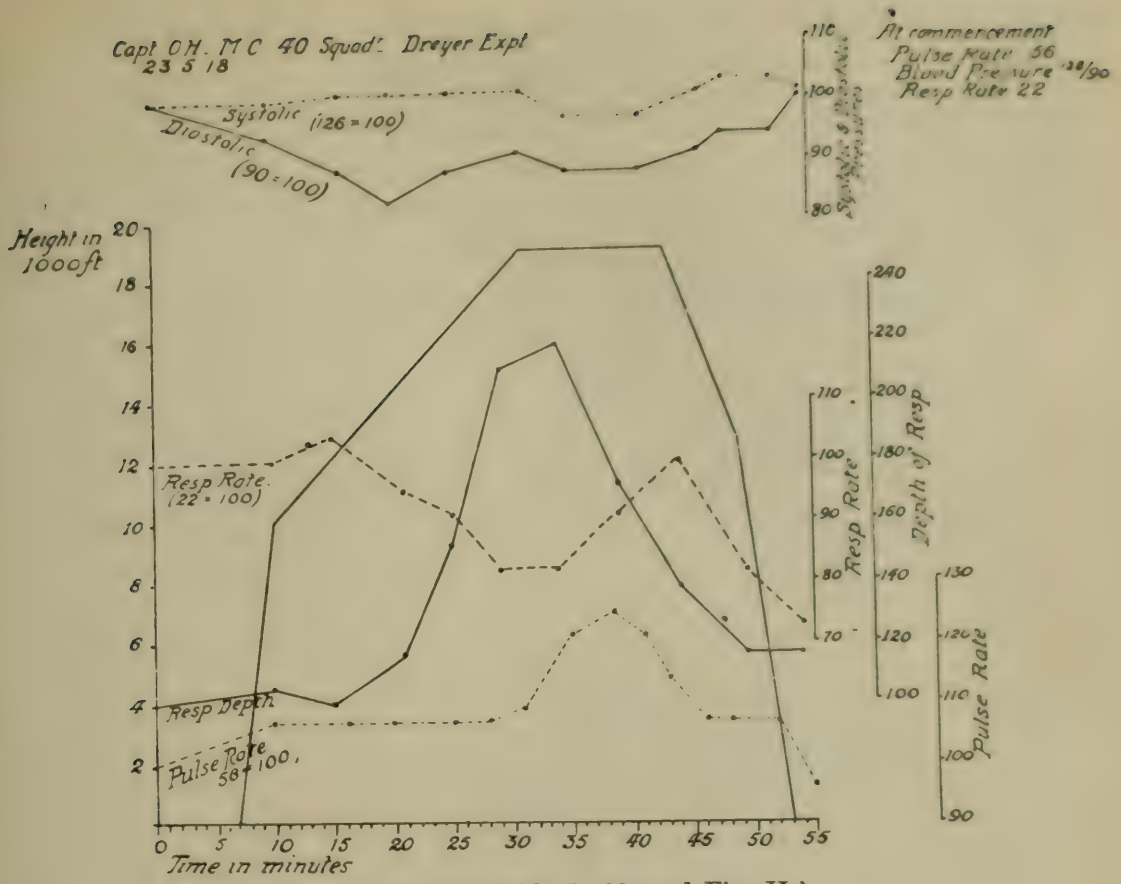


CHART 7. (See Table B, 29, and Fig. II.)

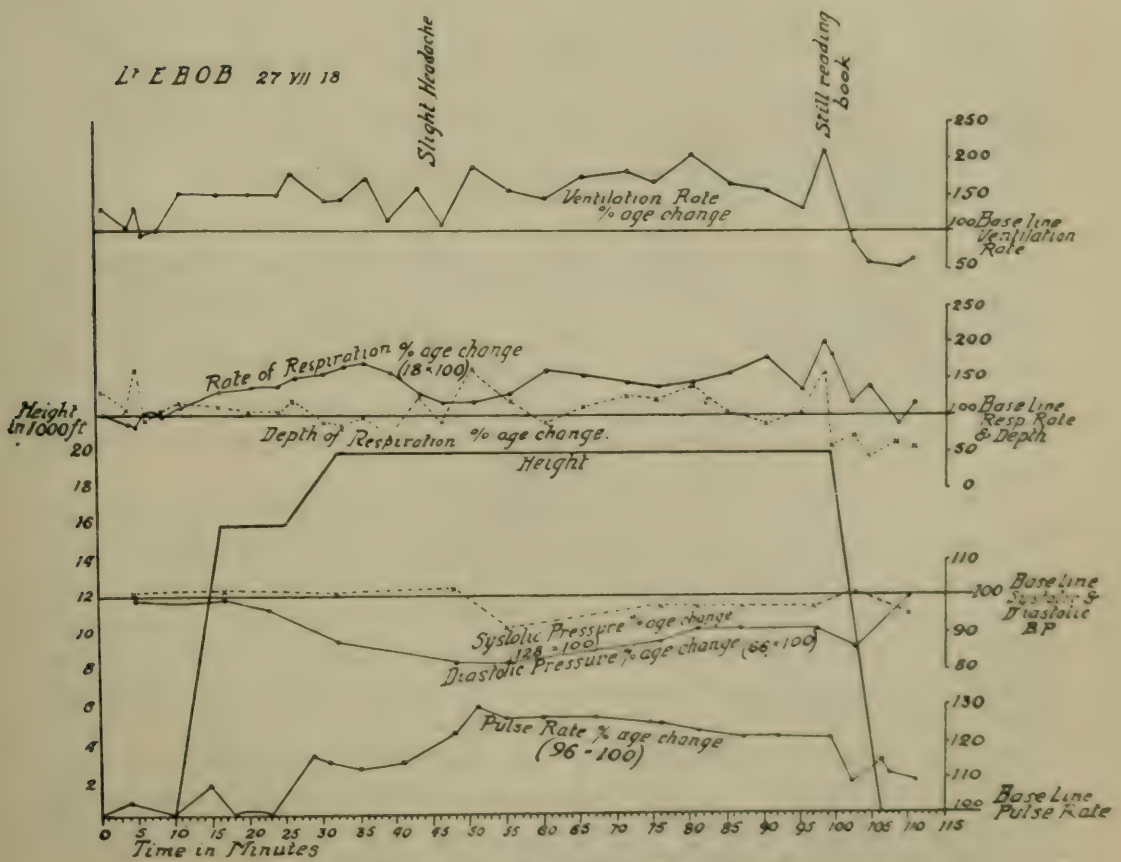


CHART 8. (See Table B, 44.)

that all individuals whose diastolic pressures are normally below this level are incapable of standing high altitudes.

The change in the haemoglobin percentage has been noted in eight cases of this class. From this small number of observations it seems that the blood is rather more dilute during the first fifteen minutes, returns to normal again after about half an hour, and later again undergoes some concentration. Possibly the delay is due to lag in the peripheral circulation.

Considerable after-effects are observed in all individuals who have

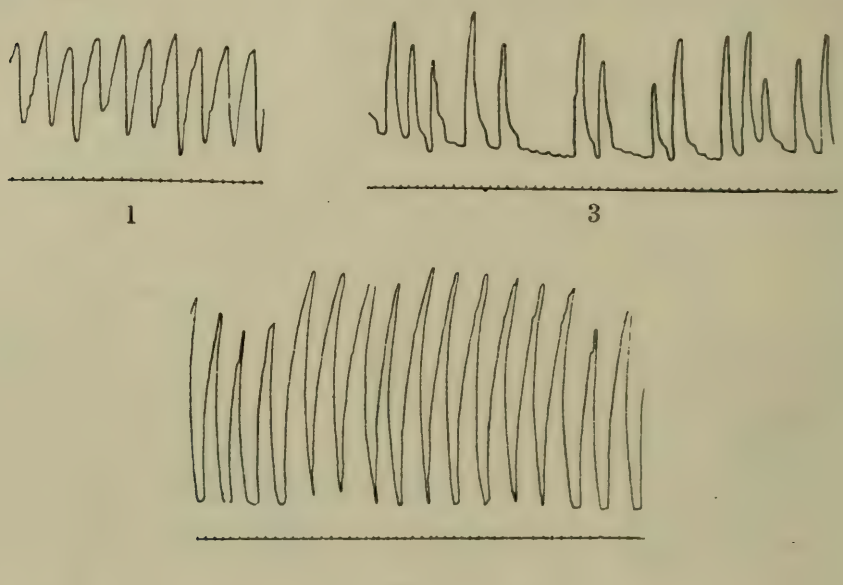


FIGURE II. (See Table B, 29, and Chart 7.)
Respiratory record, bag method.

1. Normal, ground level.
2. After 26 minutes oxygen-want, up to 19,000 feet. Depth increased. Rate slightly slowed. Very little periodicity.
3. On return to full air. Periodic breathing with apnoeic pauses.

Time-marker in this and other figures gives 1 sec. intervals.

been subjected to oxygen-want for any length of time. Respiration becomes subnormal both in rate and depth, and usually shows definite periodicity, but not so much as in bad types. Similarly the pulse-rate, systolic pressure, and pulse-pressure all become subnormal, a definite rise occurring in the diastolic pressure. Headache, when present, may persist for some hours, and there is definite fatigue both for mental and physical work.

A few comments may be made about the charts here reproduced. No. 4 is remarkable in that this officer always had a resting pulse of about 100, and yet was able to stand altitudes well, as was shown by the results here charted, and by his previous and subsequent history. Chart 5 shows a similar case, in which the blood-pressure changes are also rather abnormal, but this abnormality was probably due to the exercises he was made to do during the test; in consideration of his previous history and fast pulse these exercises were made unusually severe. Chart 7 shows a rather sluggish circulatory response and a fast initial respiratory rate. This may have been due to the fatigue from which this officer was suffering.

(b) The Bad Types.

The bad types may be classed as follows :

1. *Those in which the respiratory reaction is excessive* (Table C,

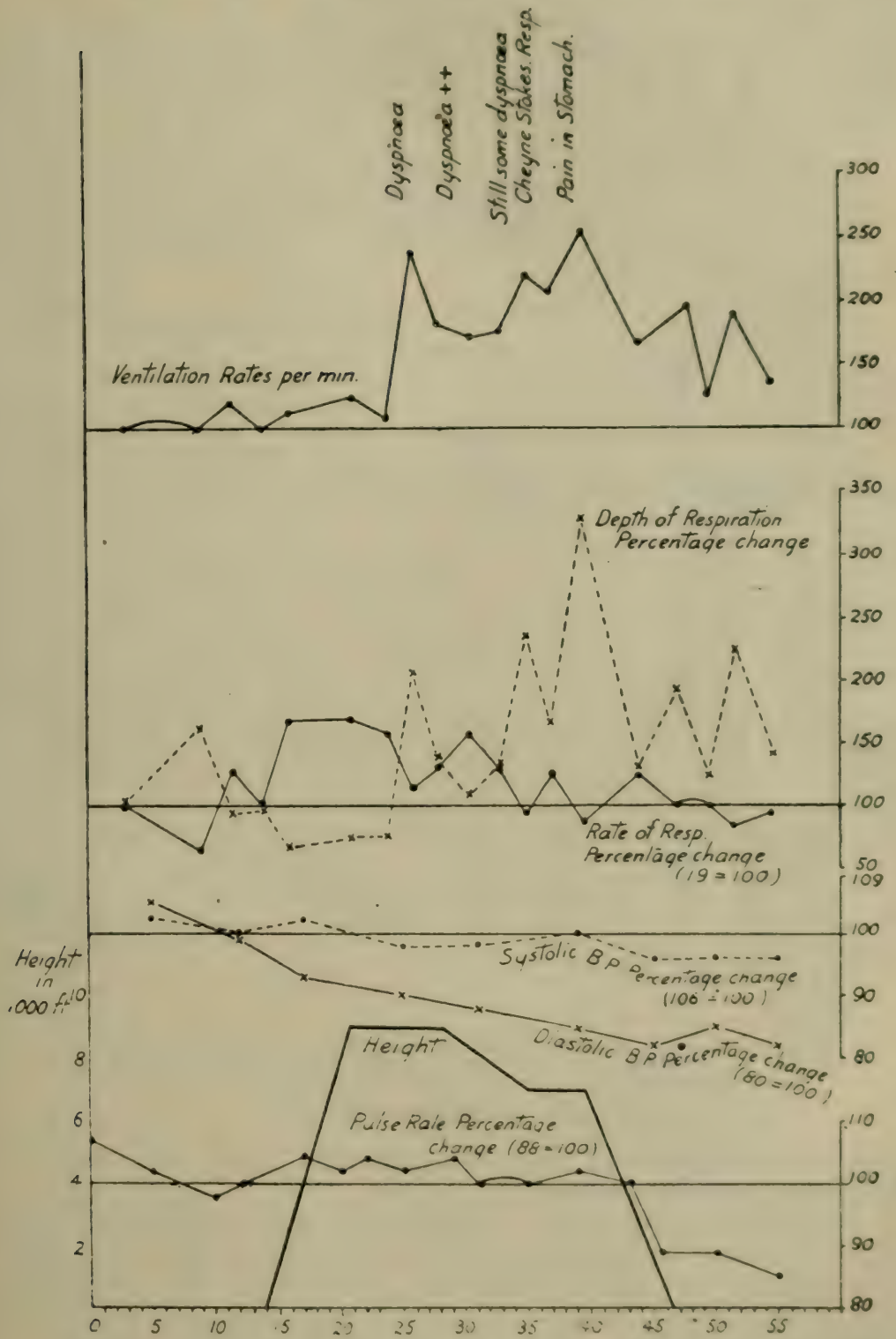


CHART 9. Lt. W. C. F. 19/7/18. (See Table C, 41.)

Charts 9 and 10 and Fig. III) causing dyspnoea, often so pronounced as to necessitate the stopping of the experiment. These individuals react at once to even a slight degree of oxygen-want, and the changes

are always associated with a subnormal vascular response. The increased ventilation is brought about both by an increase of the rate and depth of respiration, the relative importance of the two factors varying in different individuals. Towards the end of the experiment the patient gasps for breath, and breathes very deeply as well as rapidly. The pulse-rate is only temporarily increased if at all, and may show a short but definite acceleration after the nitrogen has been turned off. The systolic and diastolic

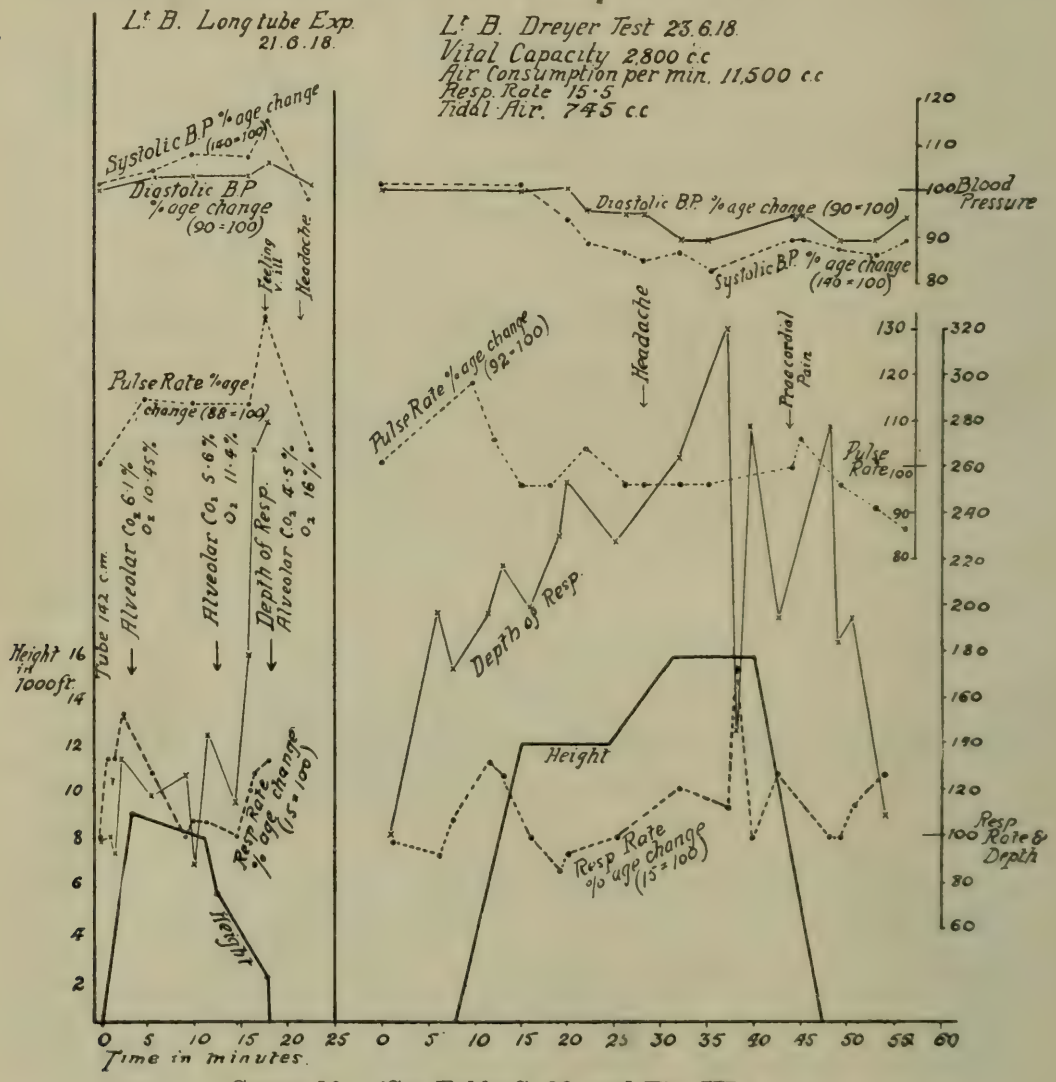


CHART 10. (See Table C, 36, and Fig. III.)

pressures have fallen in every case, while any rise in pulse-pressure reaction is usually temporary.

2. *Those in which the respiratory reaction is subnormal* (see Table D, Chart 11, and Fig. IV). In this group there is usually a temporary increase in ventilation, after which the respiration begins to fail and appears to be insufficient to compete with the abnormal conditions. Similarly the cardio-vascular response is subnormal, the increase in pulse-rate is below the average, little if any increase in pulse-pressure was seen except in one instance (43), and the systolic pressure always showed a tendency to fall. Early syncope occurred in 50 per cent. of the cases.

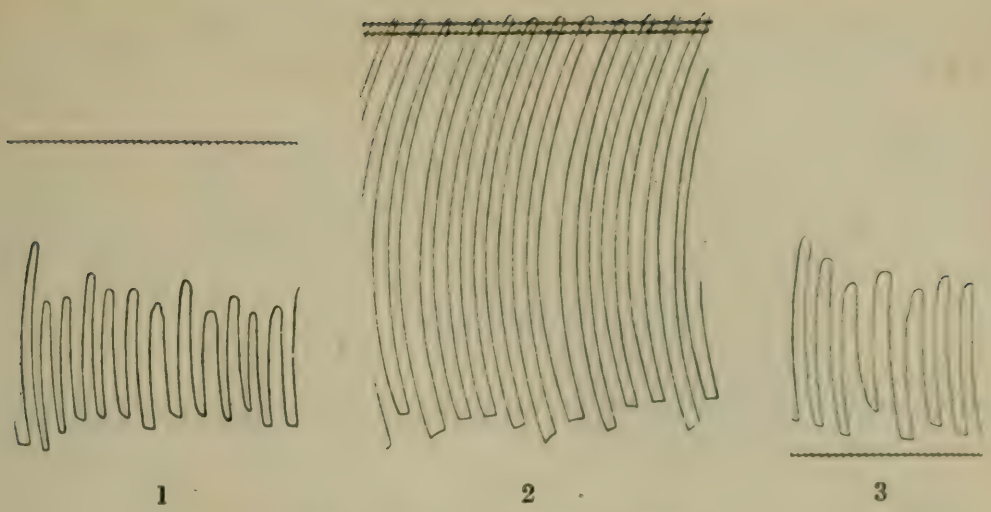


FIGURE III. (See Table C, 36.)
Respiratory record, suction method, normal tambour.

1. Normal, ground level.

2. After 27 minutes oxygen-want, reaching 15,800 feet. Little change in rate. Excessive increase in depth.

3. Seven minutes after return to ground level. Ventilation still above normal.

Note.—In tube experiment he appears to show a quite abnormal washing out of CO_2.

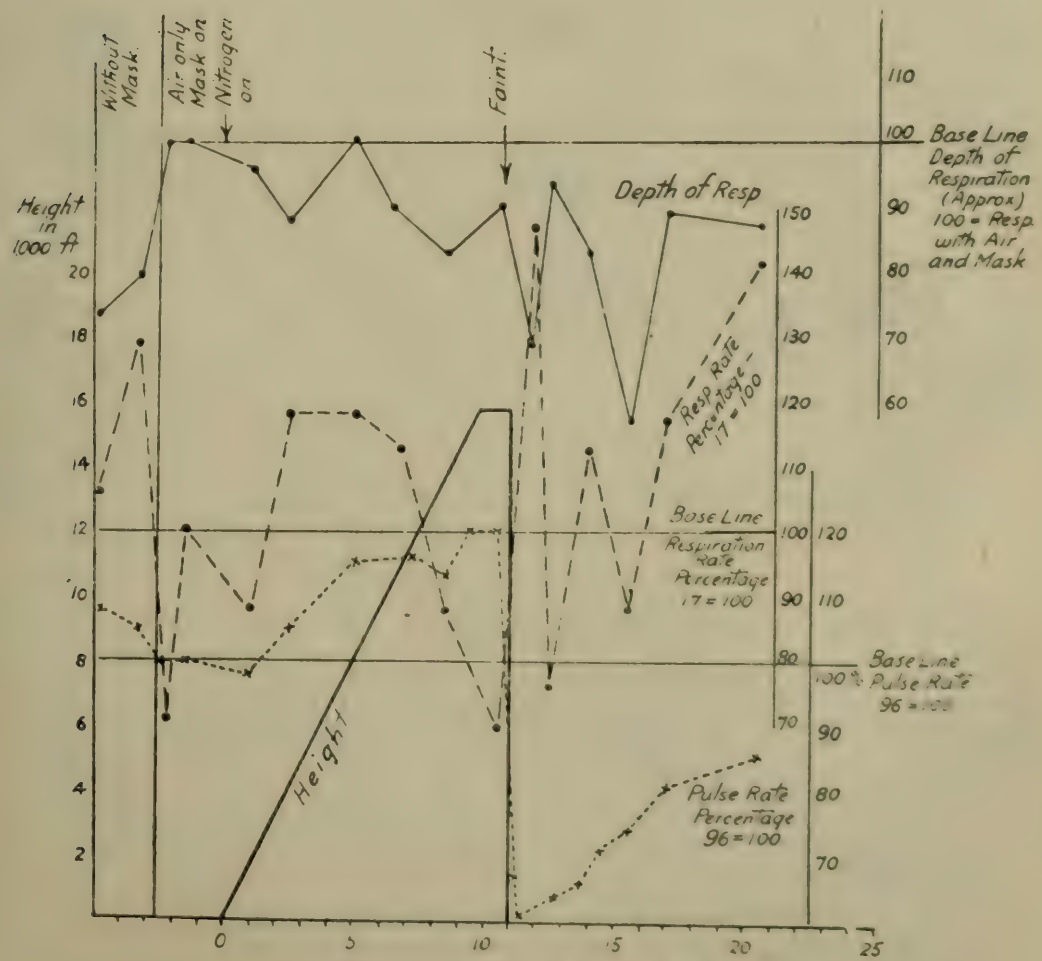


CHART 11. Lt. P. E. H. 10/4/18. (See Table D, 17.)

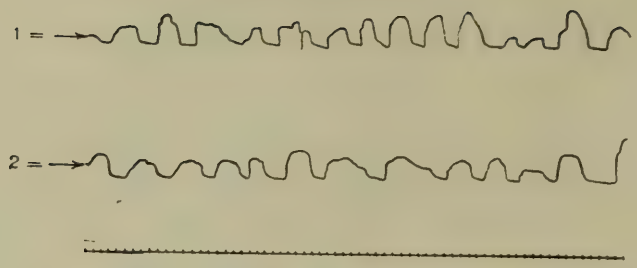


FIGURE IV. (See Table D, 43.)
Respiratory record, suction method, normal tambour.
1. Normal, ground level.
2. After 40 minutes oxygen-want, 19,500 feet. Little change in ventilation.

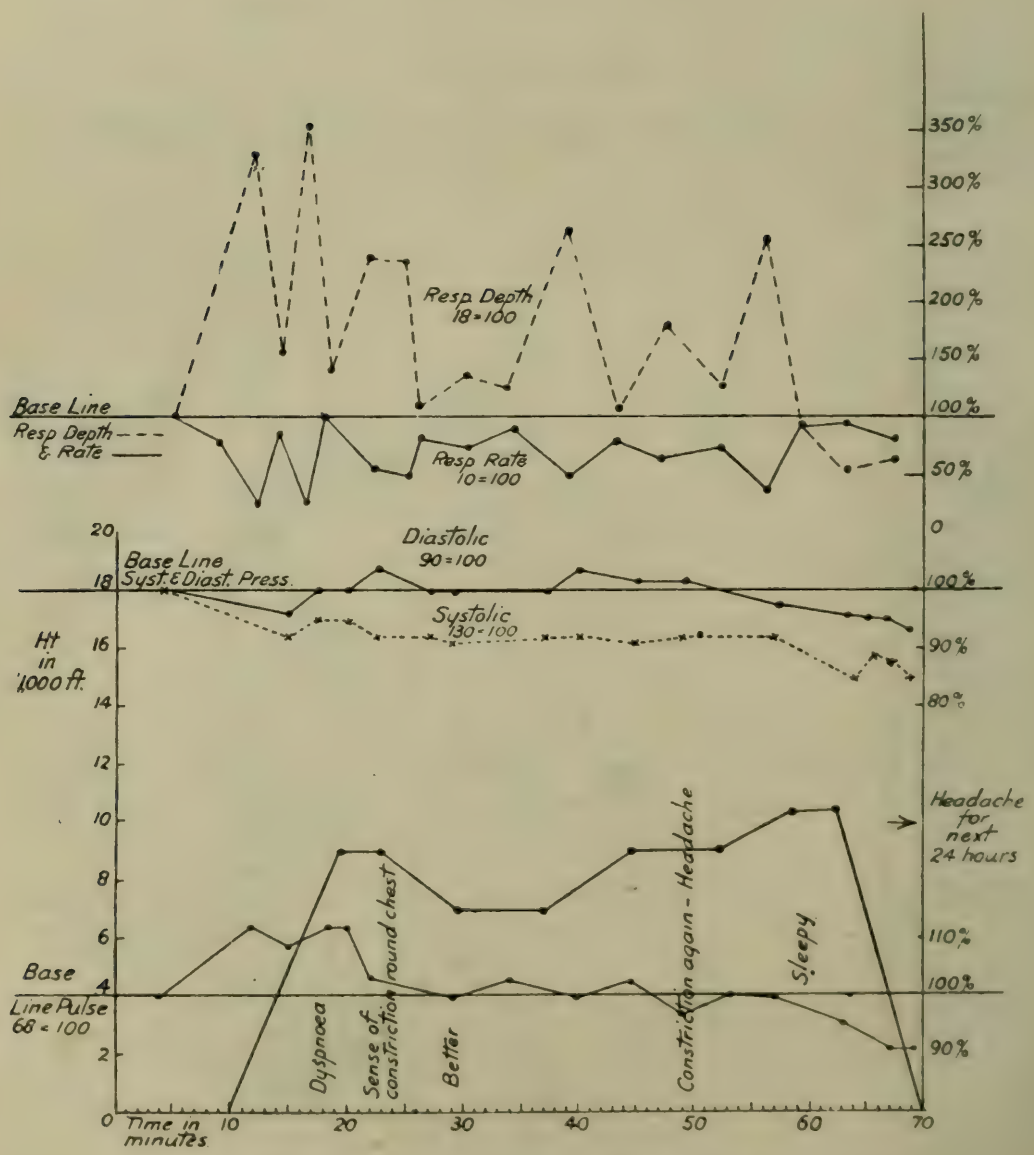


CHART 12. Lt. J. W. 19/7/18. (See Table E, 40.)

It is impossible to say whether the cardio-vascular changes are secondary to the respiratory failure or whether they have a common cause.

3. *Cases in which considerable periodicity in rate or depth of respiration was the most characteristic feature* (see Table E, Charts 12 and 13, and Figs. V and VI); in some of them the ventilation appeared to be diminished while in others it was increased, but the ill effects appeared to be due very largely to its irregularity. Associated with this group all varieties of cardio-vascular response were

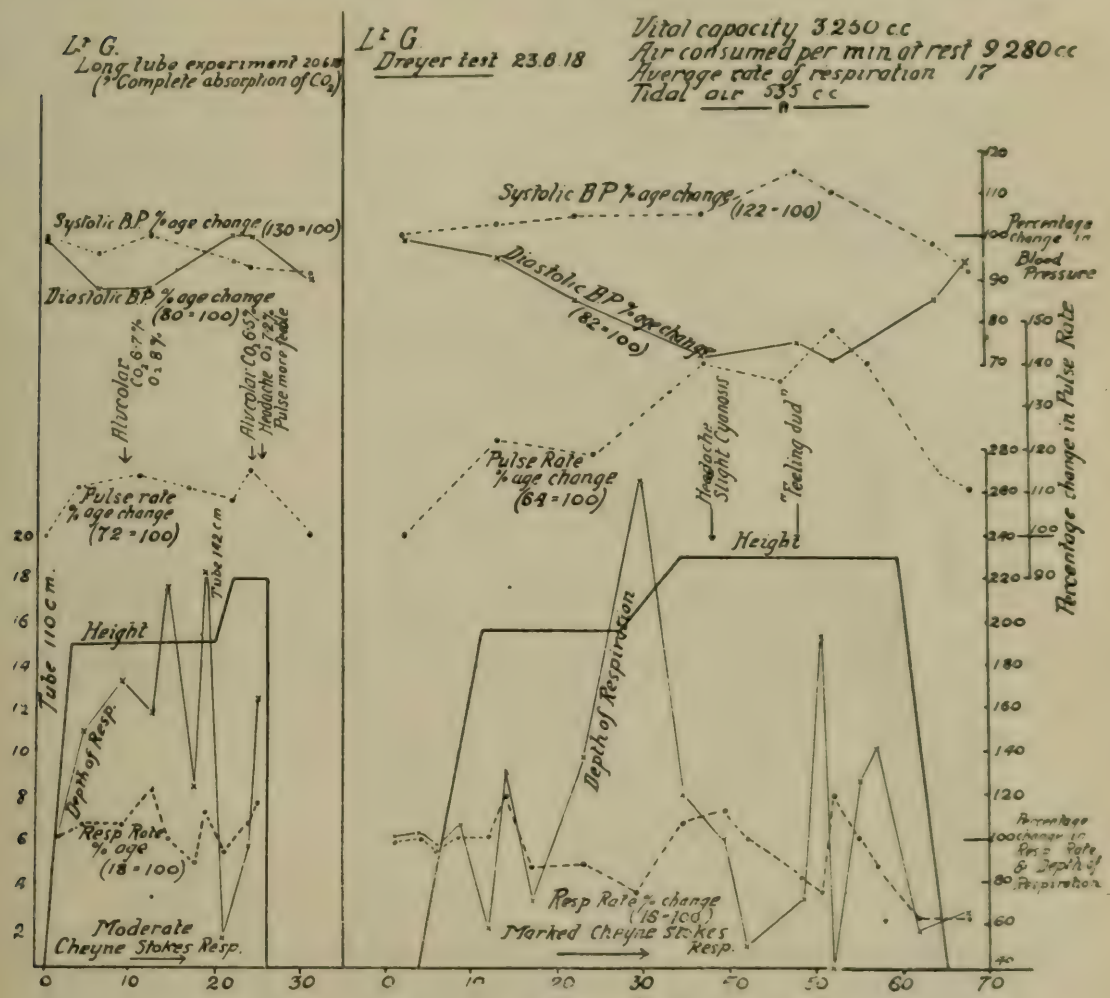
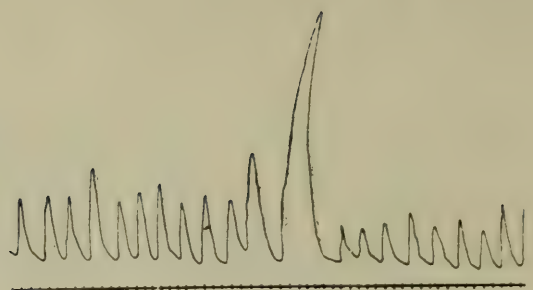


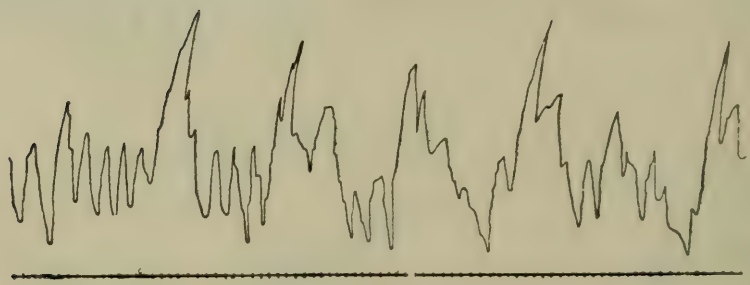
CHART 13. (See Table E, 38.)

found, and the cases might be classified under other headings according to their vascular response. In a certain number of them definite variations have been noticed in pulse-rate and blood-pressure corresponding with their apnoeic periods, during which the circulation tends to fail, and in this case it is impossible to give more than a rough approximation of the blood-pressure variations. In one case (10) the periodic breathing which came on at 18,000 feet in the air was so violent as to prevent him keeping his controls steady, and, continuing as he descended, made it very difficult for him to land.

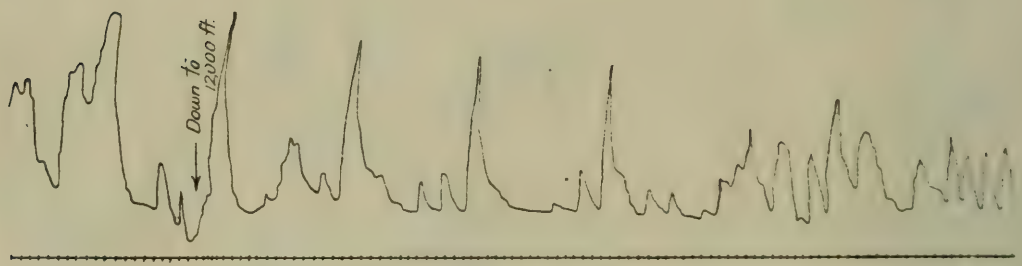
4. *Cases in which the cardio-vascular response seems to be excessive* (see Table F and Charts 14 and 15); of the five cases included



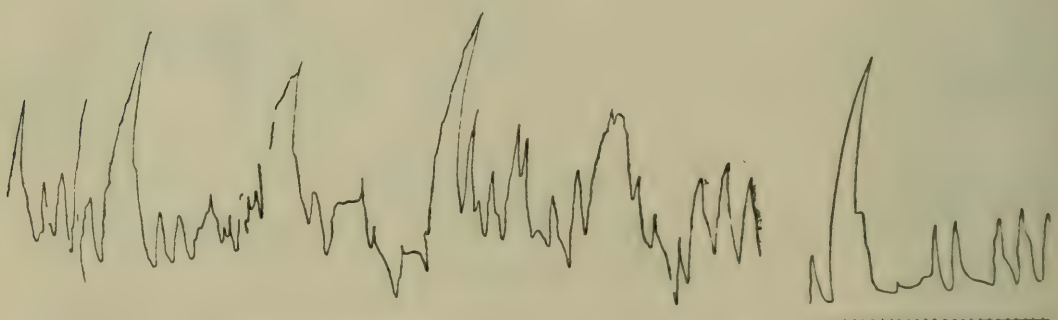
1



2



3



4 5

FIGURE V. (See Table E, 21.)
Respiratory record, bag method.

1. Normal, ground level.
2. After 20 minutes oxygen-want up to 19,000 feet. On the point of fainting.
3. Recovering from faint on being brought down to 12,000 feet.
4. Recurrence of faint five minutes later after exercise at 12,000 feet.
5. Ground level half a minute after removal of mask.

Note.—Very irregular periodicity just before syncope and similar relapse after exercise at 12,000 feet.

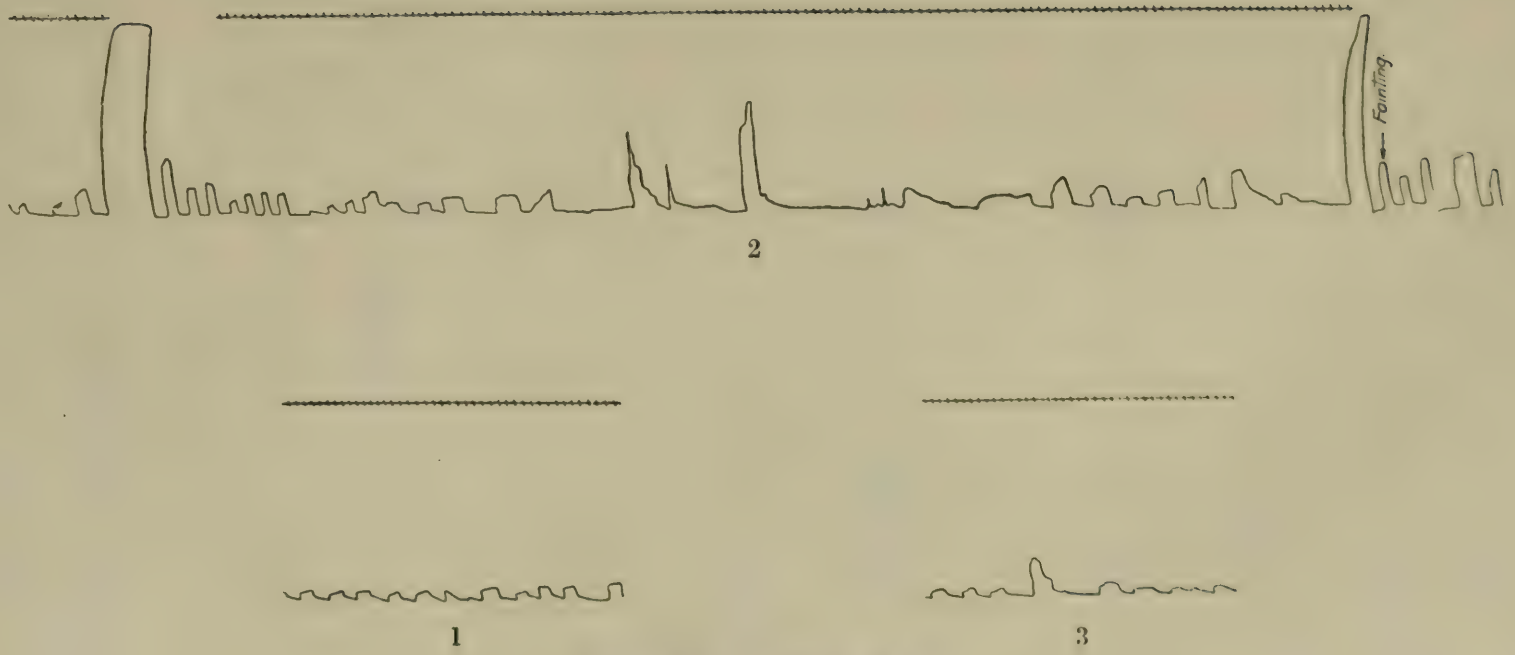


FIGURE VI. (See Table E, 45.)
Respiratory record, suction method, normal tambour.

1. Normal, ground level.
2. After 19 minutes at 17,000 feet. Very irregular periodic respiration with some apnoeic pauses.
3. Immediately on return to ground level.

in this group in only two was the respiration recorded, so that it is only possible in these two cases to state definitely that the respiratory reaction was normal. In this class the increase in pulse-rate was only slightly above the average, but the pulse-pressure was very large, showing an enormous increase unless it had been at a very high level before the experiment started. Taking the normal increase in pulse-pressure at 36 per cent., the average increase in these cases was 61 per cent. This increase was brought about by an increase in systolic as well as by a fall in diastolic pressure. It

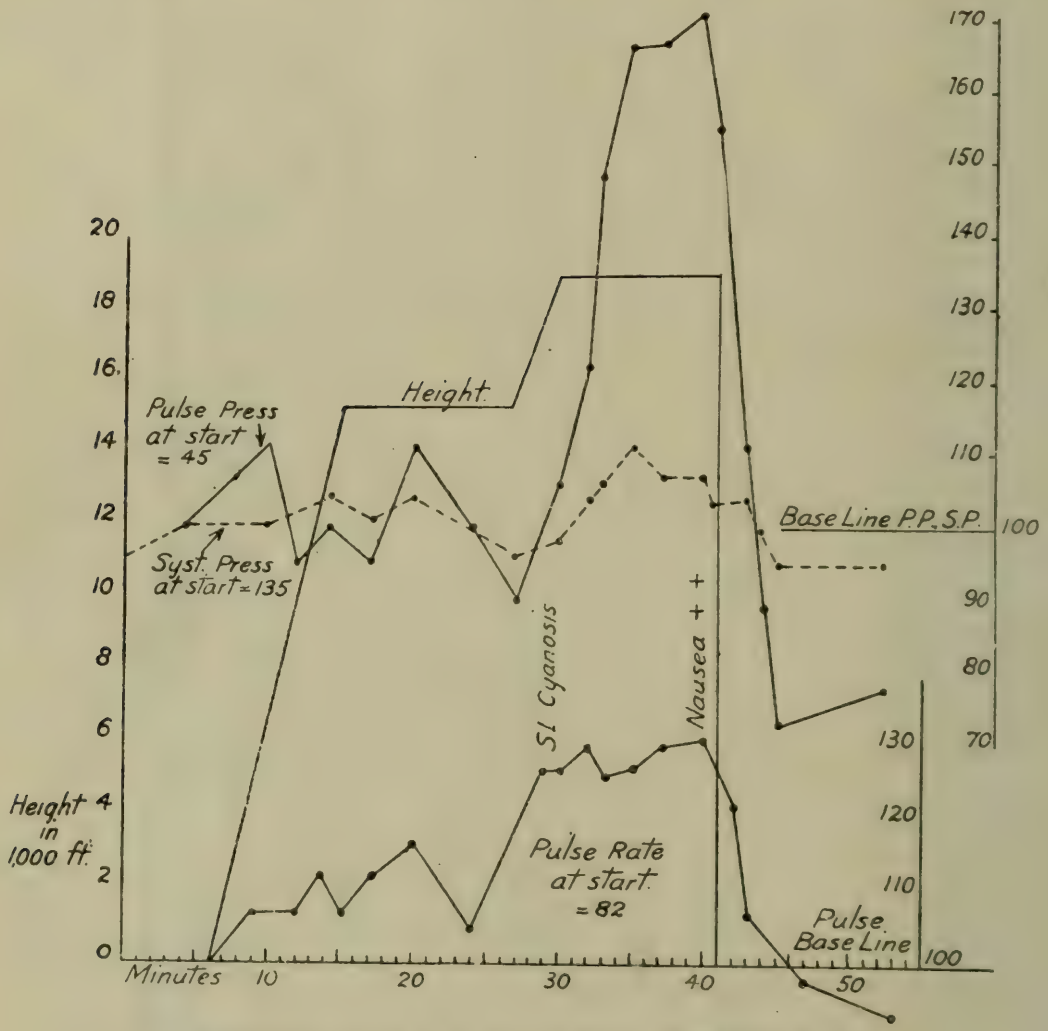


CHART 14. 2/Lt. C. J. H. 14/3/18. (See Table F, 14.)

is possible that the rise in systolic pressure occurs as an attempt to raise the mean pressure, since in these cases the diastolic pressure falls to a very low level.

5. *Cases in which the cardio-vascular response seems to be subnormal* (see Table G and Charts 16 and 17); of eight cases in this group the respiration was recorded in five, so that it can be definitely stated that this subnormal reaction may be seen associated with a normal respiratory response. On the other hand, cases with a similar cardio-vascular reaction are included in groups 1 and 2, and some occur in group 3. The average increase in pulse-rate was only

13 per cent., but the change in pulse-pressure is not so definitely subnormal. The systolic pressure remained fairly constant. Of the symptoms sleepiness was the most prominent, often associated with little distress, and in some cases scarcely realized by the patient himself.

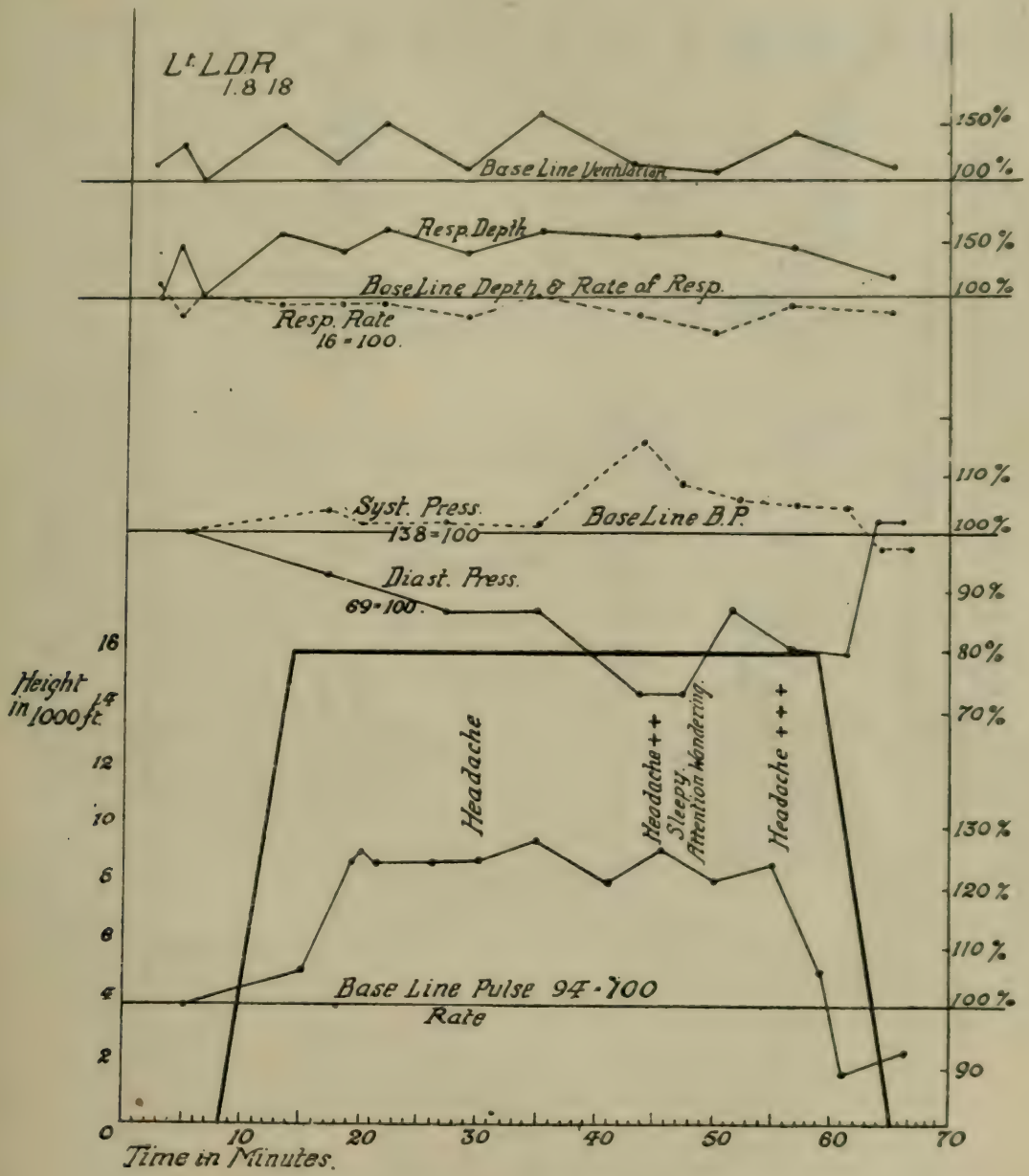


CHART 15. (See Table F, 48.)

This type of case appears to react to altitude by a general lowering of activity, and these symptoms must be regarded of considerable importance because the individuals do not realize how inefficient they are when doing any kind of work on high-flying machines.

Syncope. Actual syncope was not often observed, as the experiment was usually stopped before it supervened. It was noted in eight cases, including cases of every kind of respiratory reaction, but in six out of the eight cases the cardio-vascular response was definitely subnormal from the commencement. Where the blood-

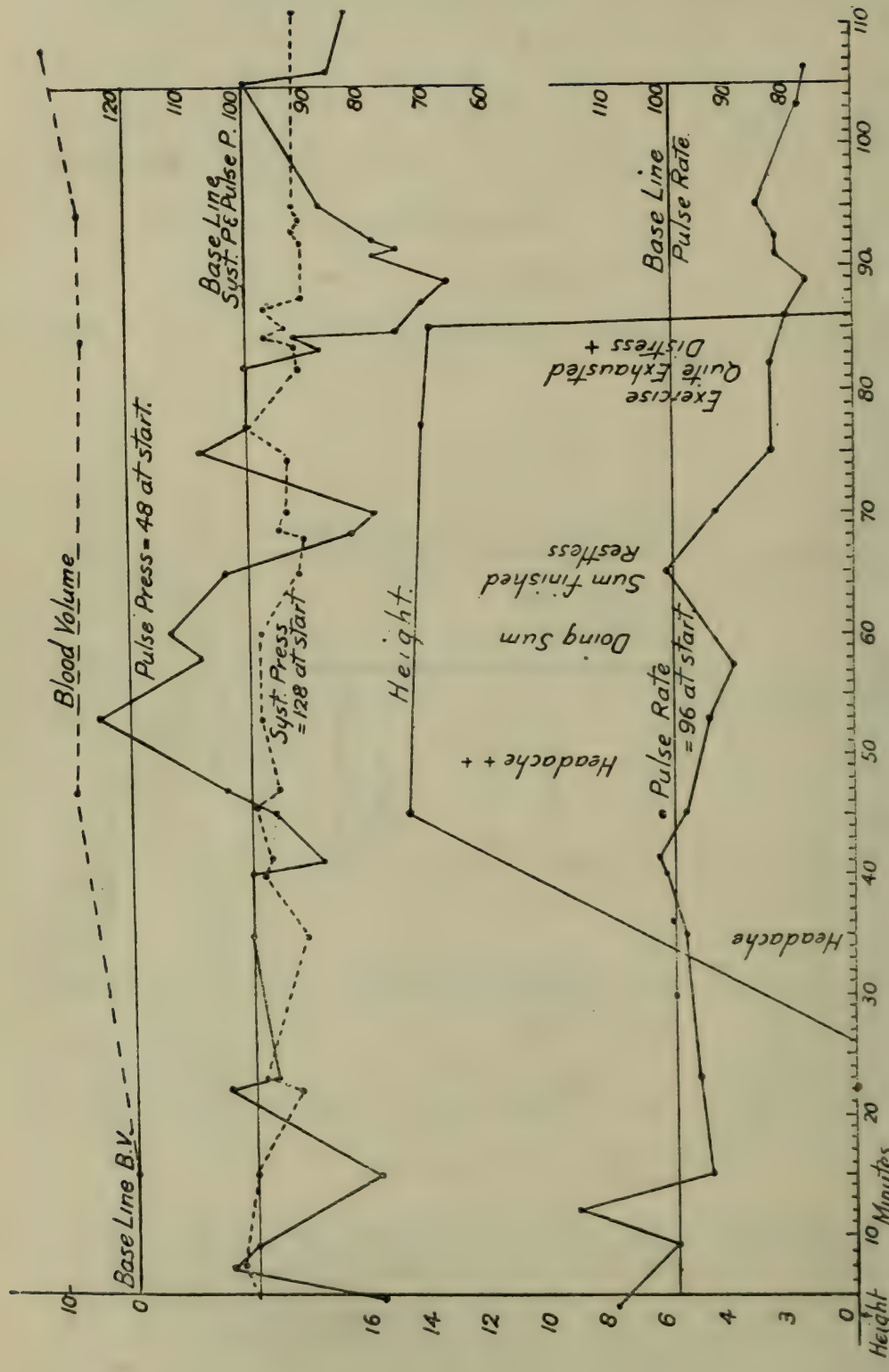


CHART 16. 2/Lt. C. H. H. (See Table G, 12.)

pressure was taken shortly before syncope occurred the pulse-pressure was found to be definitely decreasing (the diastolic pressure often rising), followed somewhat later by a fall in systolic pressure. At the onset of syncope a rapid fall of both pressures was observed, but quick recovery was seen as soon as the patient was given fresh air.

Syncope in these cases with low oxygen tensions seemed to be due to cardiac failure, since it was often associated with a slow thready pulse, preceded by a period of gradually lessening pulse-

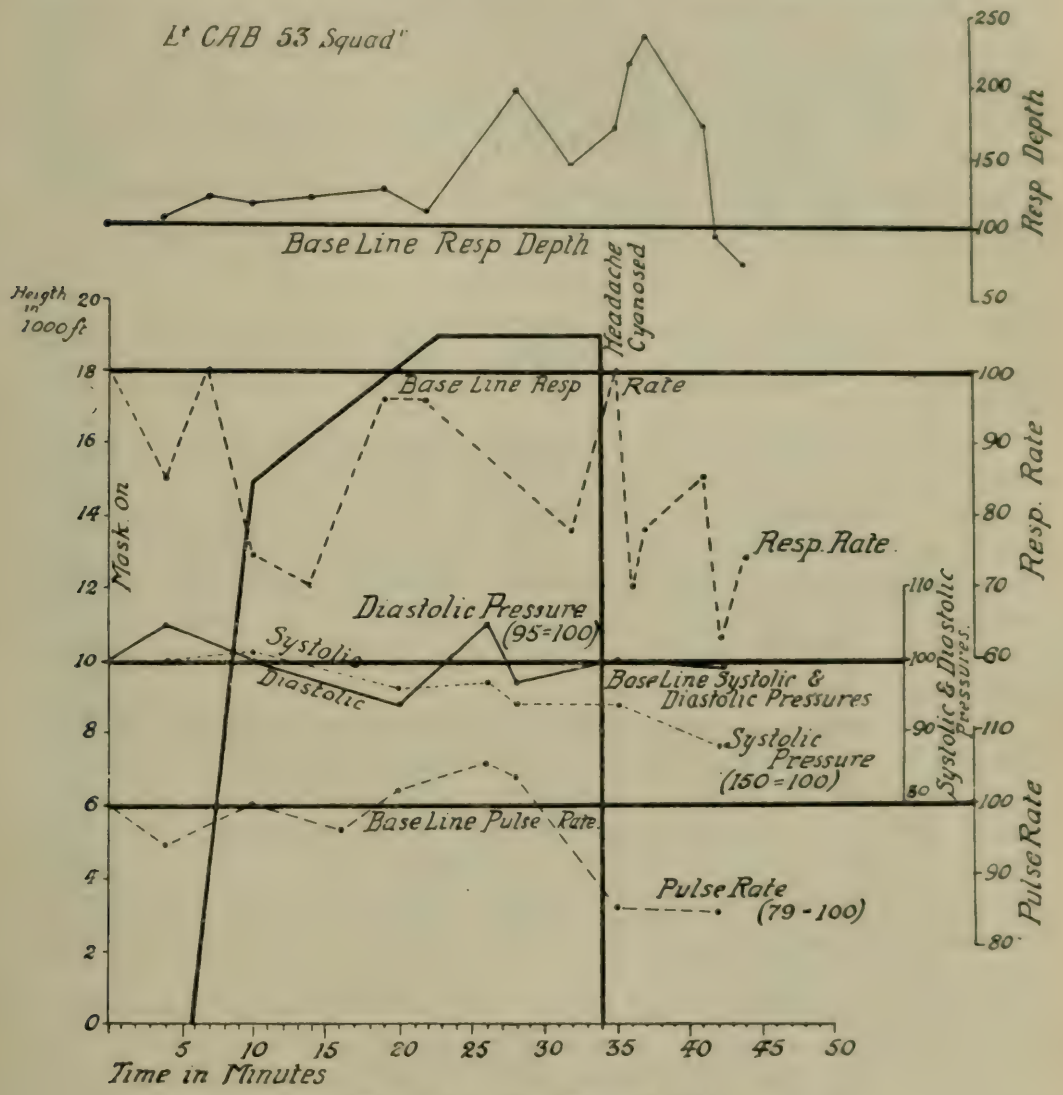


CHART 17. (See Table G 27.)

pressure and raised diastolic pressure. The condition was quite different from the faint of a nervous boy as the result of a medical examination (e.g. taking of blood-pressure), where we can confirm the observations of Cotton and Lewis (5), since we also found in these cases a marked slowing of the pulse accompanied by a great fall in both systolic and diastolic blood-pressures.

Arbitrary Arrangement into Groups. The bad types have been divided into various groups for convenience of description, and to some extent these distinctions are definite. It must, however, be recognized that many cases could be classed into more than one

group, while in other cases it is extremely difficult to decide what is the chief cause of failure. In the cases showing periodic respiration it is often very difficult to settle whether the ventilation is above or below normal, and probably many cases included in this group should properly belong to either the excessive or subnormal respiratory types. Though big differences in sensitiveness to oxygen-want occur, it is impossible to explain them in all cases by an abnormal response of the nervous centres to chemical stimuli, since the cardio-vascular and respiratory responses do not always run parallel. Very few observations on blood-volume changes have so far been made on the bad types, but a few are included in the tables. From these it will be seen that the blood-concentration seen in the first cases did not occur or if present was not maintained. It is possible that blood-volume changes may be of great importance, but time has not allowed any further investigation of this point.

E. Conclusions.

1. The Dreyer method of testing the capacity of airmen to stand diminished oxygen tension is of great practical value and the best so far devised.

2. The normal reaction to oxygen-want corresponding to about 18,000 feet is an increase in pulse-rate of about 30 per cent., of pulse-pressure of about 36 per cent., and after about half an hour some degree of blood-concentration. The pulse-pressure rise is brought about mainly by a fall in diastolic pressure, while the systolic pressure in the absence of mental or physical work is only slightly raised. The respiration is slowed and deepened, increasing the ventilation about 50 per cent.

3. Where failure to withstand oxygen-want occurs it may be associated with an abnormal respiratory response, excessive, subnormal, or periodic, while in other cases the cardio-vascular system seems mainly at fault. In these failure appears to be caused by a subnormal reaction except in a few instances with a very big pulse-pressure in which the diastolic pressure appears to fall to a level so low as to be harmful. It is doubtful whether blood-concentration occurs in cases liable to failure.

4. The symptoms from which the patient has suffered when actually in the air may be reproduced by the Dreyer method, but it is most important to realize that a patient who claims to be perfectly fit at high altitudes may in reality be quite inefficient; sleepiness is the most common symptom and yet is often not recognized by the airman.

5. Symptoms of oxygen-want may even occur in rare cases at altitudes below 10,000 feet, although the individual may appear perfectly healthy.

We are both indebted to Lt.-Col. J. L. Birley for his keen interest and help in the preparation of this paper.

Appendix

In revising this paper before it is reprinted (in 1920) I (H. C. B.) have thought it best to add to it a few extra charts, selected out of a large number which had to be omitted in the original publication owing to the difficulties of reproduction

during the war. After the publication of the paper an additional eleven tests were made, which must also be alluded to ; I do not propose to describe them all in detail since the complete record of the changes observed is not now available, so that only the exceptional cases will be discussed at all fully. I also want to take this opportunity to refer briefly to the conclusions drawn by other workers, who have recently published results obtained under similar conditions, especially as much of this work appeared at about the same date as our own.

Of the 11 additional tests, 5 had marked periodic breathing with a cardio-vascular response that was more or less subnormal; 3 gave a simple subnormal cardio-vascular response, and 2 a subnormal cardio-vascular response, accompanied in one case by an excessive respiratory response with great increase in the depth of respiration, and in the other with a very shallow but very rapid respiration. The other man gave a normal response. These two last cases are instructive in some respects and consequently protocols are given in some detail, supplying also a fuller indication of the methods employed, though these cases have not been so carefully worked out as the previous ones, and their respiratory records are not now available for accurate analysis.

(1) Lt. S. S. G. 17 hours as observer on day bombers. Shortness of breath and sleepiness at altitudes. Poor physique. Results of respiratory tests given in Report No. 9, Table II, 26 (p. 195).

Time.	*Approximate Altitude.*	*Pulse Rate.*	*Blood Pressure.*	*Remarks.*
2.20	Sea level			Mask on.
2.30	.. .. ..	84		
2.35	.. .. ..	90		
2.38	.. .. ..		116/72	
2.40	.. .. ..	86		
2.41	.. ..		116/72	
2.42	Rising			Nitrogen turned on.
2.45	↓	78		
2.47	↓		116 to 108 / 70 to 60	Swinging of blood-pressure, pulse-rate also irregular. Respiration rate increased.
2.49	12,000 ft.	92		
2.51	.. .. ..	92		
2.53	Rising			
2.55	↓		? 106/64	
2.58	↓	103	? 116/60	Headache, sleepy.
3.0	15,000 ft.	102		
3.10	.. ..		? 106/94	
3.12	.. .. ..	91		
3.15	Rising			
3.19	.. .. ..	96		More sleepy.
3.20	.. ..		? 106/60	
3.21	.. .. ..	96		Headache bad. Still reading book Not distressed.
3.26	.. .. ..	94		Put down book. Respiration rate now 52, very shallow and periodic in depth.
3.30	.. ..		? 110/64	Can hardly keep eyes open. Very dull and sleepy.
3.32	Coming down ↓			Respirations still quicker and becoming periodic also in rate Rate reaching 72 at times.
3.36	Nearly sea level	72	96/68	Respirations shallow and periodic in rate and depth.

Comments. Increase in respiratory rate very marked. Shallow rapid respiration (28) at normal oxygen tensions and this increased at lower tensions. Very unstable blood-pressure showing big fluctuations, and never staying constant. Increase in pulse-rate up to 20 per cent., but not maintained, and very little change in pulse-pressure. Patient never cyanosed during the test.

This patient, therefore, shows a subnormal cardio-vascular response with an extreme degree of rapid shallow respiration similar to that described by Haldane, Meakins, and Priestley (6).

(2) Lt. G. S. An officer of good type with 110 hours day bombing to his

credit. Found flights trying and attributed it himself to nerve strain. When found fit to fly at altitudes he transferred to infantry at his own request. Physique very good. Results of respiratory tests given in Report No. 9, Table I, 22.

Time.	*Approximate Altitude.*	*Pulse Rate.*	*Blood Pressure.*	*Remarks.*
2.25	.. .. ..	..	..	Mask on.
2.30	.. .. ..	80	124/80	
2.35	.. .. ..	80	124/80	
2.40	Rising v. rapidly ↓			
2.50	24,500 ft.	112	128/64	Very cyanosed. Headache. Stops reading.
2.53	.. .. ..	120		Respiration deepened. Rate steady
2.54	Coming down ↓			
3.0	18,600 ft.			
3.5	.. .. ..	81	118/66	Less cyanosed. Some headache. Reading again. Deep respirations. Rate steady.
3.10	Rising			
3.13	↓	90		Very cyanosed. Restless. Stops
3.17	24,000 ft.		128/64	reading. Deeper respiration.
3.18	.. .. ..	100		
3.24	.. .. ..	114	146/60	B.P. showing fluctuations.
3.31	Coming down			
3.33	.. .. ..	96		
3.35	.. .. ..	..	120/80	
3.37	.. .. ..	84		
3.43	.. .. ..	84	120/82	

Comments. This case illustrates the immensely rapid changes in oxygen tension that can be endured by a fit man. The 'altitudes' were confirmed by analysis of four samples of the gas mixtures in this case. The respirations were deepened and the rate was throughout steady or slightly slowed. The cardio-vascular response is of interest in that the pulse-rate slowed when the patient was brought to an altitude of 18,600 from a temporary 24,500, so that carrying him to the greater height had perhaps increased his rate of adaptation to the ordinary test level. Also later when he was returned to an altitude of 24,000 the blood-pressure reaction which had been normal in character changed to one with an excessive increase in pulse-pressure judged by tests at lower levels, with an increased systolic, a lowered diastolic pressure, and a commencing instability of both pressures. This suggests that the character of the response is to some extent dependent on the altitude at which the individual is tested relative to the greatest height to which he can readily adapt himself, emphasizing still further the arbitrary character of any division into groups. Possibly the reaction seen at the highest limits tolerated is one in which adrenalin plays a part, corresponding with the results obtained on cats by Kellaway (7).

General Discussion. Various types of respiratory response have been described in this paper and confirmation of Haldane's work on Cheyne-Stokes respiration has been obtained. On the other hand, only a small percentage of the individuals examined showed any great increase in the respiratory rate on exposure to low oxygen tensions, a type of response relatively common among the subjects used by Haldane and his co-workers (6) and (8). In this difference we are in agreement with American workers, who also found the normal reaction to be a deepening of the respiration with the maintenance of a normal rate even on exposure to quite low oxygen tensions (9) and (10).

Haldane and his co-workers in the last paper quoted report a change of the reaction of the urine in an alkaline direction in adaptation to low oxygen tensions. Samples of urine were taken after two of our tests (Tables B 5 and E 6) and were examined for us by Dr. H. Maclean, to whom we are much indebted. In both cases the reaction was found at the extreme range of normal limits of alkalinity. We were unable to continue these determinations through pressure of routine work, and we did not realize their significance at the time, but so far as they go, they bring forward confirmatory evidence of Haldane's theories, and so are mentioned here.

Very similar variations in the character of the cardio-vascular response to low oxygen tensions are reported by American workers using the re-breathing test (9), (10), and (11). Only one considerable difference is noticeable, since these workers found that in the good response the diastolic pressure remained steady or was slightly raised, while we have found it lowered, and this, too, in spite of the fact that they also used an auscultatory method for determining the diastolic pressure. The contrast probably depends on the different conditions of the two tests; whereas the American method gradually reduced the oxygen in the air breathed to 8 or 7 per cent., this process requiring 25 to 30 minutes, we reduced the oxygen percentage at once usually to 12 per cent., and often to 10 per cent., and maintained the patient at this level for 40 minutes or longer. In the rebreathing test the subjects were only exposed to oxygen percentages below 12 per cent. for some 10 minutes, and a fall in the diastolic pressure in many cases in the terminal part of the test is reported. Reference to Charts 2, 5, and 6 of the American Air Service Medical Manual shows that this fall commonly commenced at 11 to 12 per cent. of oxygen, so that there is really an agreement with our results, since all our normal cases were maintained at or below this percentage. The case they show in Chart 7 in the same manual is also instructive as to the importance of this fall in diastolic pressure. They note that 'he compensates in some way but does not do it in the usual way'. He commenced with a pulse-rate of 90 to 96 and a blood-pressure of 110/68 to 118/70; with 13 per cent. oxygen the pulse-rate is 87 and the blood-pressure 130/70, and with 7·5 per cent. oxygen the pulse-rate is 90 and blood-pressure 126/58. This case showed then an increase in pulse-pressure of 51 per cent., and may have been even greater since the fluctuations of pressure before the test suggest that the 'normal' pressure may have been altered by excitement. That adaptation can occur with an increase in pulse-pressure though raising of the systolic or lowering of the diastolic pressure seems to be sustained by their results as well as ours, always excepting cases where the diastolic pressure falls to a seriously low level.

More recently Lutz and Schneider (12) have published further data using low pressure chamber, rebreathing apparatus and Dreyer's method, and they too have found a lowering of the diastolic pressure and an increased pulse-pressure, when the oxygen tension is held at a low level. Gregg, Lutz, and Schneider (13) record haemoglobin changes similar to ours. Lutz and Schneider also describe a later diminution of the increase in the pulse-pressure as adaptation to the low oxygen tensions is developed; our results certainly favour such a conclusion (see Charts 3, 6, and 7) but we did not have enough evidence to make any definite statements. The same workers find syncope preceded by a fall in both diastolic and systolic pressures; this we have found also in four of our cases of syncope, but in three others in which the pressure changes were more continuously observed, the earliest sign appeared to be a decrease in pulse-pressure generally accompanied by a rise of diastolic pressure, which then develops into the fall. Examples of this may be given shortly.

Lt. H. (Table G 12) commenced with a blood-pressure of 128/80; at 14,600 ft. after 30 minutes his pressure was 126/66, after 51 minutes 128/80; he was then given some exercise and after this felt very faint and the last blood-pressure record preceding the fall was 120/84. In another patient (one of the later additional cases, Lt. D.) the original blood-pressure was 110/70 and the pulse-rate 80. After 28 minutes at 15,000 ft. it was 114/60 and the pulse-rate 90 to 96 and very irregular. He had praecordial pain; after 31 minutes it was 106/56 with a pulse-rate of 88 and after 38 minutes he began to faint. The last record before the fall was rate 88 and very irregular, blood-pressure 106/80.

The agreement between the results obtained by these American workers and our own is the more remarkable in that their tests were performed on recruits, and ours on officers admitted to hospital suffering from slight staleness, severe fatigue, or other conditions due to flying.

Some apology is necessary for the expression of oxygen tensions as changes in altitude or in oxygen percentage. No accurate record was kept of tensions since we were concerned with gross differences between individuals, and to facilitate the disposal of patients all our results were expressed in altitudes. Haldane and his co-workers (8) suggest that any oxygen tension produced by dilution with nitrogen is a severer test than the same tension produced by a lowering of the atmospheric pressure, owing to the nitrogen hindering the diffusion of oxygen. If this is so, then our patients were exposed to a severer test than

is indicated by the charts, but it is remarkable how accurately experienced pilots were able to gauge the 'height' to which they were exposed. Haldane in the same paper also states that owing to the effect of the CO_2 content of blood on the dissociation curve of oxyhaemoglobin, adaptation to low oxygen tensions cannot be brought about by mere changes in the respiration and circulation rate. This does not seem to be supported by our results, since the efficiency of the individual at low oxygen tensions ran more or less parallel with the reactions of the circulatory and respiratory systems. We have drawn attention to the slowing of the pulse, as the blood concentration commences, and this concentration may perhaps be associated with the secretion of alkali by the kidney, but the initial response is effected by the circulation and respiration. Possibly in spite of a more rapid circulation and a lowered CO_2 content, the reaction of the blood in certain tissues is maintained normal by other factors, so that the oxygen is given up readily.

That the more slowly developed methods of adaptation are the more effective, however, seems to be supported by the improvement in the symptoms, which often occurs after about 30 minutes, and by the fact that the severity of the test is very much lessened, if the oxygen percentage is lowered more slowly.

The charts have been expressed in percentage changes rather than in absolute figures. This has received some criticism, and has objections in that the actual value of the mean pressure is of considerable importance and is not easily deduced from the charts. But this method was adopted in order to compare men of different initial conditions—for instance subject 2 (Table B) had a rise of pulse-rate of 16 beats per minute, while subject 20 (Table B) had an increase of 33 beats per minute; but on the percentage basis the difference was only one between 31 per cent. and 35 per cent., and this seemed most nearly to express the real difference. A further examination of the figures obtained, a consideration of the evidence they give of the vascular changes involved and of the probable effect on the circulation rate, is in course of production and will be published later.

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TABLE B. DREYER'S METHOD—NORMAL RESPONSE

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pressure Pulse Change.*	*Blood Volume Change. (Time in Min. from Time N turned on)*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
1. B. E. B. 11–2–18	Just completed 300 hrs. flying at 18–20,000 ft. Now a little stale. Physically very fit.	35′	21,000	68 ↓ 84 (24 %)		—	—	100 ↓ 102 (16′) ↓ 100 (45′)	—	—	—	—	—	Hands and feet cold—fairly fit. Due for H. E
2. F. N. J. 13–2–18	M.O. Healthy. Has never flown.	25′	23,000	64 ↓ 84 (31 %)		—	—	—	—	—	—	—	+	Pale—faint towards close.
3. G. P. 14–2–18	Scout pilot. 56 hrs. Noticed slight dyspnoea above 12,000 ft.	40′	21,500	60 ↓ 80 (33 %)		—	—	100 ↓ 100 (26′) ↓ 102 (45′)	Deepening with periodic breathing above 19,000 ft. (No record.)	—	—	—	—	Has done very well since, but mostly low work. Awarded M.C
4. T. T. C. 22–2–18	Observer. Extreme coldness of extremities above 12,000 ft.	46′	21,000	84 ↓ 108 (29 %)	—	—	—	100 ↓ 97 (44′) ↓ 96 (53′)	—	—	+	—	—	No coldness of extremities during experiment. To duty.
5. H. C. B. 24–2–18	M.O. Had not flown. Healthy.	84′	18,000 later 24,500	68 ↓ 104 (53 %)	113 ↓ 129 (14 %)	75 ↓ 83 ↓ 75	38 ↓ 50 (34 %)	100 ↓ 101 (14′) ↓ 100 (51′) ↓ 93 (68′) ↓ 93 (94′)	Deepening. (No record.)	+	—	—	+ at the greater height.	At 18,000 ft. felt fitter after first hour. This corresponded with time of blood concentration. (See Chart 1.)

TABLE B (*continued*)

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change. (Time in Min. from Time N turned on.)*	*Respiration Change.*	Symptoms. *Head-ache.*	*Sleepi-ness.*	*Dys-pnoea.*	*Syn-cope.*	*Remarks.*
7. J. H. H. 1-3-18	Pilot. 30 hrs. Suffering slightly from nervous shock. No symptoms in air. ? Aggravated by oxygen-want.	43′	18,500	54 ↓ 88 (63 %)	132 ↓ 146 (11 %) ↓ 132	82 ↓ 64	50 ↓ 72 (44 %) ↓ 68 (36 %)	100 ↓ 100 (35′) ↓ 106 (51′) ↓ 100 (62′)	Deepening. (No record.)	+	+	—	—	Given sick leave but not returned.
11. H. M. S. 7-3-18	Observer. Malarial history. Persistent tachycardia. Feels quite fit. Bad physique.	69′	19,600	98 ↓ 129 (32 %)	128 ↓ 140 (9 %)	80 ↓ 63 ↓ 70	48 ↓ 72 (50 %)	100 ↓ 98 (34′) ↓ 94 (60′) ↓ 95 (85′)	—	+	—	—	—	Subsequently did very stout work in Bristol Fighter Sqdn. for many months. Since killed in action. Note rise in pulse-rate from high level.
16. C. F. C. 4-4-18	Scout pilot. 125 hrs. Recently fainted after landing.	45′	19,000	68 ↓ 86 (26 %)	110 ↓ 126 (15 %)	70 ↓ 60	40 ↓ 60 (50 %)	100 ↓ 100 (41′)	—	—	—	—	—	Very tired on admission to hospital. BP = 95/70. Recovered. Sent to England.

20. C. J. M. 20–4–18	Observer. 200 hrs. Excitable Irishman. Previously turned down by Comms. Board as Pilot.	53′	20,000	96 ↓ 129 (35 %)	142 ↓ 142 ↓ 125 (after exercise)	85 ↓ 70 ↓ 90 (after exercise)	62 ↓ 72 (16 %) ↓ 35 (after exercise)	100 ↓ 104 (16′) ↓ 99 (41′) ↓ 101 (58′)	Deepening showing mild periodicity. 40% increase in ventilation.	—	—	—	—	Just completed long spell of heavy work in Bristol Fighters. Subsequently passed fit as Pilot by Comms. Board. Note rise in pulse-rate from high level.
29. O. H. 23–5–18	Scout pilot. 190 hrs. Recent severe attack of pain left scapular region at 18,000 ft. Shaky on landing.	46′	19,000	56 ↓ 70 (25 %)	128 ↓ 132	90 ↓ 75 ↓ 85	38 ↓ 55 (45 %)	—	Depth increased. Rate slowed. No periodicity. Ventilation about 60 % increase.	—	–	—	—	A good deal of insomnia recently. Very tired. Fine type. To England. (See Fig. II.)
37. J. B. 23–6–18	Pilot. Passed unfit for flying out here 6 mons. previously. Oxygen-want at 9,000 ft. Immature.	49′	19,000	72 ↓ 94 (30 %)	110 ↓ 112	70 ↓ 60	40 ↓ 52 (30 %)	—	Rate slight increase. Depth increased, moderate periodic breathing. Ventilation about 50 % increase.	—	—	—	—	Been at various infantry schools with much exercise. Passed fit below 10,000 ft.
42. R. J. T.W.	Pilot. No flying in France. Height neurosis.	66′	10,500	68 ↓ 74 (9 %)	108 ↓ 114	60 ↓ 64	48 ↓ 52 (8 %)	—	Changes slight.	Slight	—	—	—	Sent to medical flight.
44. E.B.O.B.	Pilot. 80 hrs. on Bristols. Praecordial pain and dyspnoea above 10,000 ft.	87′	20,000	96 ↓ 124 (29 %)	128 ↓ 130 ↓ 124	66 ↓ 54	62 ↓ 76 (23 %) ↓ 64	—	Increased rate. Ventilation about 60 % increase.	Slight	—	—	—	Excitable: probably temperamentally unsuited for high flying.

TABLE C. DREYER'S METHOD—PERIODIC RESPIRATORY RESPONSE

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change.*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
3. C. W. P. 12-3-18	Observer. 35 hrs. Fainted at 15,000 ft. Recovered and fainted again at 16,700 ft. Remembers little till he landed.	21′	19,200	78 ↓ 94 (20 %)	122 ↓ 115	85 ↓ 68 ↓ 85	37 ↓ 55 (49 %) ↓ 33	—	Rate increased over 200%. (No record.)	+	+	++	+	Constant swinging of diastolic pressure under test. On ground normal reaction of B.P. to exercise much exaggerated. Passed fit night bombers only. Did excellent work 4½ months and then sent home to learn to fly.
36. C. R. B.	Pilot Scouts. 40 hrs. Definite O_2 want at 10,000 ft. Thought to be malingering by C.O.	39′	12,000 ↓ 15,800	92 ↓ 105 ↓ 88	140 ↓ 115	90 ↓ 80	50 ↓ 35	—	Rate and depth both increased. (Depth ++).	+	—	++	—	Excitable type. A fine pilot. Passed fit below 10,000 ft. (See Fig. III.)
41. W. C. F.	Pilot. 3 hours in France. Concussion 3 months ago. O_2 want at 9,000 ft.	32′	9,000 ↓ 7,000	88 ↓ 92 ↓ 90	106 ↓ 108 ↓ 104	80 ↓ 68	26 ↓ 38	—	Depth much increased. Rate slightly increased.	—	—	++	—	Very poor physique. Eustachian obstruction. Thickened M.T. and impaired movement of ossicles. Sent to England. (See Chart 2.)
49. H. J. F.	Pilot. 70 hours in France. Praecordial pain at 18,000 ft. Shortness of breath on ground lately.	26′	18,600	96 ↓ 110 ↓ 104	118 ↓ 106	72 ↓ 58	46 ↓ 56 ↓ 48	—	Depth much increased. Rate showing little change except periodicity.	+	—	+	—	Restless. D.A.H. type of pulse. Found permanently unfit for flying.

TABLE D. DREYER'S METHOD—SUBNORMAL RESPIRATORY RESPONSE

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change.*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
17. P. E. H. 10/4/18	Pilot. 50 hrs. in France. Fainted at 14,000 ft. Recovered at 4,000.	11′	15,800	96 ↓ 115 (20 %)	—	—	—	—	Depth decreased. Rate increased at first, but later below normal. Marked periodicity in after-re-action.	—	—	—	+	Polygraph record of pulse-rate changes. Excitable type, flushes easily. Also tested on Flack's Bag. (See Chart 3.) Boarded permanently unfit flying; fit G.S.
18. G. E. H. McE.	Pilot Scouts. Very fine type. 215 hours in France. Gassed Nov. 1915. Occasional attacks of asthma since. Now very tired.	32′	18,000	69 ↓ 84 (22 %)	118 ↓ 118 ↓ 100 ↓ 88	85 ↓ 85 ↓ 70 ↓ 60	33 ↓ 33 ↓ 30 ↓ 20	—	Slower rate. Depth increased at first, but soon becoming subnormal. Very incomplete record	—	—	—	+	Unexpected sudden onset of fainting. Note fall of systolic and pulse pressures indicating onset of syncope. Has been home and out again, and is now (21/7/18) getting symptoms of O_2 want. 1/8/18. Missing. Has brought down 40 to 50 E.A.

TABLE D (*continued*)

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change.*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
26. T R. D.	Pilot. 8 hours in France. O_2 want at 10,000 ft. Shortness of breath.	25′	17,300	74 ↓ 79 (7 %)	120 ↓ 100	85 ↓ 60	35 ↓ 40	—	Diminution in depth without increase in rate. Some periodicity.	+	—	—	—	Found permanently unfit for flying. Fit G.S. on ground.
33. C.K.H.D.	Pilot. 500 hours in France. Now doing special work. Very tired and not standing height as well as he did.	27′	17,000	68 ↓ 82 (21 %)	100 ↓ 88	65 ↓ 55	35 ↓ 33	—	Rate and depth unchanged. Irregular rather than periodic.	+	—	—	+	
34. A. N. O.		59′	18,500	58 ↓ 72 (24 %)	110 ↓ 98	60 ↓ 58 ↓ 64	50 ↓ 34	—	Rate not increased. Transient increase depth, then subnormal. Some periodicity, especially in after reaction.	—	++	—	—	
43. J. H. G.	Observer. 50 hrs. in France. Recent syncope in air after a fight. Previous to this had been getting symptoms of O_2 want.	46′	15,000 ↓ 20,000	68 ↓ 78 (16 %)	110 ↓ 115 ↓ 108	80 ↓ 50	30 ↓ 58	—	Rate subnormal. Depth increased periodically, but insufficient to balance diminished rate.	+	+	—	—	Passed fit night bombers only (See Fig IV.)

TABLE E. DREYER'S METHOD—PERIODIC RESPIRATORY RESPONSE

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change (Time in brackets.)*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
10 A. S. K.	Pilot (test). 220 hours in France. At 18,000 ft. had attack of severe periodic breathing, so that machine got out of control. On landing had to be helped from machine.	57′	18,000	60 ↓ 81 (35 %)	126 ↓ 122	84 ↓ 68	42 ↓ 56 (33 %)	100 ↓ 102 (30′) ↓ 100 (50′) ↓ 94 (73′)	Excessive periodic breathing after 25 min., lasting for 20 min. after return to full air.	+	+	+ +	—	Has since done very good work in DH, squadron, but always uses oxygen and has had no recurrence of attack. Awarded M.C. Since wounded.
19. E. A. M.	Pilot. 10 hours in France. When diving rapidly from 18,000-9,000 ft. felt sick and vomited at 2,000 ft. Very dizzy. Crashed into another machine on landing.	30′	18,000	80 ↓ 100 (25 %)	114 ↓ 120	78 ↓ 56	36 ↓ 62 (72 %)	—	Excessive periodic changes in rate, less periodicity in depth. Ventilation decreased.	+	—	+	—	Returned to duty. M.O. asked to watch him. Did well, but since missing.
21. C. A. M.	Pilot. 10 hours in France. Gasps for breath above 12 000 ft. Has nearly fainted 3 times at 18,000 ft.	29′	18,000	78 ↓ 92 (18 %)	130 ↓ 132	78 ↓ 56	53 ↓ 76 (46 %)		Subnormal, with periodicity very irregular at onset of syncope.	—	—	—	+	To fly below 10,000 ft (See Fig. V.)

TABLE E (*continued*)

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change. (Time in brackets.)*	*Respiration Change*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
23. G. H. R.	Pilot. 13 hours in France. Aged 18. Very immature. Severe attack pleurodynia afterflying. Had been working 11 hours a day for a year in factory before joining.	35′	15,000	74 ↓ 92 ↓ 72	85 ↓ 88–104 (swinging)	65 ↓ 58–70 (swinging)	20 ↓ ?	—	Rate slowed; marked periodicity in depth, lasting 25 min. after end of experiment.	+	—	+	—	Pulse on admission was 55 sitting, 100 standing, and 112 exercise: typical of exhaustion. During test remarkable swing of blood-pressure—never constant. To England.
25. A.H.S.M.	Pilot Scouts. 46 hours. Symptoms oxygen-want at 11,000 ft.	12′	13,000	84 ↓ 80 ↓ (126 on coming down)	148 ↓ 135	95 ↓ 85	50 ↓ 50	—	Rate and depth increased, especi lly towards end. Definite periodicity (not shown in Chart).	—	—	++	—	Passed for below 10,000 ft. Has done mostly ground work since discharge; still has marked aversion to flying above 1,000 ft. ? Neurosis. Is going to infantry at own request.
28. C. R. B.	Pilot. 15 hours in France. Dived from 10,000 to 8,000 ft. very fast. Lost consciousness, but recovered at 500 ft.	38′	18,000	68 ↓ 98 (44 %)	120 ↓ 125 ↓ 106	85 ↓ 68 ↓ 70	35 ↓ 57 ↓ 36	—	Rate slowed; periodicity and diminution of ventilation.	—	—	+	—	Went back to duty, but now always auto-inflates his ears when diving. No recurrence of syncope. Readmitted later and sent home for enucleation of tonsils.

31. W. H. R.	Scout pilot. 100 hours in France. 6 weeks' headache, dyspnoea, and vertigo at 12–15,000 ft.	60′	19,500 (max.)	63 ↓ 83 (31 %)	148 ↓ 138	80 ↓ 54	68 ↓ 84 (24 %)	—	Rate slowed; periodicity + in depth. Ventilation diminished.	—	+	—	—	Instructed always to use O_2. Since broken down completely. To Canada.
38. F. R. G.	Pilot. 12 hours in France. Symptoms of O_2 want above 10,000 ft.	61′	19,000	64 ↓ 95 (4 8 %)	122 ↓ 140 (15 %)	82 ↓ 58	40 ↓ 78 (95 %)	—	Periodicity in rate and depth, especially depth.	+	+	—	—	To have oxygen set fixed if necessary.
39. Sgt. S.	Observer. 38 hours in France. Symptoms of O_2 want at 17,000 ft	55′	10,500	69 ↓ 88 ↓ 77	104 ↓ 120 ↓ 114	38 ↓ 86 ↓ 74	38 ↓ 34 ↓ 40	—	Periodicity in depth.	+	+	—	—	Periodic breathing and mental dullness in spite of low height. To fly below 10,000 ft.
40. J. W.	Pilot. 30 hours in France. Symptoms of O_2 want above 9,000 ft.	60′	10,500	69 ↓ 76 ↓ 68	130 ↓ 120	90 ↓ 94 ↓ 88	40 ↓ 24 ↓ 32	—	Rate very subnormal. Periodicity in depth. Long apnoeic periods.	+	+	+ early stages	—	Praecordial pain. To Medical Squadron. (See Chart 4.)
45. W. H. G.	Pilot. 5 hours in France. Symptoms of O_2 want at 13,000 ft. Dizziness, inclined to syncope. Has Eustachian obstruction.	22′	17,000	68 ↓ 96 (41 %)	118 ↓ 124 ↓ 95	76 ↓ 80 ↓ 50	42 ↓ 44 ↓ 45	—	Rate fast, 26 per min., showing little change in depth. Marked periodicity in depth.	—	+	—	+	Extra systoles disappearing during test. Sent to England. Eustachian obstruction to be treated. Then fit night bombers only. (See Fig. VI.)

TABLE E (*continued*)

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change. (Time in brackets.)*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
46. J. H. G.	Pilot. 5 hours in France. History of symptoms of O_2 want in England at 9,000 ft. Dizziness, headache, &c.	36′	10,500 ↓ 16,000	64 ↓ 77 (20 %)	116 ↓ 114 ↓ 112	78 ↓ 74 ↓ 92	38 ↓ 42 ↓ 20	—	Periodicity in rate and depth, changing to respiration of Class 1.	+	—	+	+ early	At 10,500 periodicity of rate, deep slow respirations, ventilation changing at 16,000 to very rapid deep breathing. Recommended for sea-planes.
47. B. P.	Pilot. ½ hour in France. History of concussion in September 1917. O_2 want above 5,000 ft., severe at 10,000 ft.	76′	11,000 ↓ 16,000	68 ↓ 76 (12 %)	138 ↓ 146 ↓ 136	66 ↓ 58 ↓ 62	72 ↓ 88 ↓ 74	—	Periodicity in rate and depth, with diminution of ventilation.	—	++	—	—	Abdominal pain and some nausea. Big respiratory variations in blood-pressure. Periodic breathing started at 11,000 ft. Disability associated with temperamental unfitness. Unlikely to be of any value in air.

TABLE F. DREYER'S METHOD—EXCESSIVE CARDIO-VASCULAR RESPONSE

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change. (Time in brackets.)*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
6. W. J. S.	Observer. 55 hrs. France. Cannot work at altitude without oxygen.	61′	20,000	84 ↓ 106 (26 %)	133 ↓ 125 ↓ 147	88 ↓ 65 ↓ 58	45 ↓ 60 ↓ 89 (98 %)	100 ↓ 102 (55′)	Not recorded. Deep breathing and dyspnoea.	++	+	+	—	Returned to duty, always to use oxygen. Did quite well. Missing 3 months later.
14. C. J. H.	Pilot Scouts. 70 hrs. Vomiting in air above 12,000 ft. during previous month. Very fatigued on landing.	37′	19,000	82 ↓ 107 (30 %)	135 ↓ 150	90 ↓ 68	45 ↓ 77 (71 %)	—	Not recorded.	—	—	—	—	Sudden nausea at end of experiment necessitating removal of mask. Passed fit as test pilot below 10,000 ft. (See Chart 5.)
15. J. G. B.	Pilot Scouts. 170 hours. France. Symptoms of oxygen-want at 15,000 ft. and over.	46′	19,000	88 ↓ 132 (50 %)	125 ↓ 137	75 ↓ 50	50 ↓ 82 (64 %)	—	Not recorded.	+	—	—	—	Recommended for training on 2-seater, preferably night bomber.
24. H.E.F S.	Pilot. 33 hrs. France. Oxygen-want at 14,000 ft Very sleepy.	37′	19,000	64 ↓ 92 (44 %)	122 ↓ 140	40 ↓ ? < 40 ↓ 45	82 ↓ > 10 (22 %) ↓ 95	—	Slowed and deepened with some periodicity.	—	+	—	—	Extremely low diastolic pressure all time while in hospital. Very young, sent to England to develop.
48 L. D. R.	Pilot. Not flown in France. Concussed 6 months ago, only done light flying since. Feels ill above 5,000 ft.	50′	16,000	96 ↓ 120 (30 %)	140 ↓ 160	68 ↓ 50 ↓ 60	72 ↓ 110 (53 %) ↓ 86	—	Slowed and deepened, but ventilation moderately increased. Only slight periodicity.	++	+	—	—	Should never have been sent overseas. Good type, but immature. Sent to England. Note pulse pressure.

TABLE G. DREYER'S METHOD—SUBNORMAL CARDIO-VASCULAR RESPONSE

Number and Name.	*History.*	*Duration of Test.*	*Height of Test in Feet.*	*Pulse Rate Change.*	*Systolic Pressure Change.*	*Diastolic Pressure Change.*	*Pulse Pressure Change.*	*Blood Volume Change. (Time in brackets.)*	*Respiration Change.*	Symptoms. *Headache.*	*Sleepiness.*	*Dyspnoea.*	*Syncope.*	*Remarks.*
8. K. W. C.	Pilot. 20 hrs. France. Dizziness at any altitude. Landing headaches. Short of breath on ground. Tachycardia.	72′	19,000	97 ↓ 113 (17 %)	134 ↓ 140	76 ↓ 64 ↓ 78	58 ↓ 70 (20 %) ↓ 50	100 ↓ 96 (38′) ↓ 95 (63′) ↓ 101 (80′) ↓ 98 (98′) ↓ 105(118′) ↓ 105(118′)	No record. Some periodic deep breathing.	—	+	+	—	Disability partly temperamental. Permanently unfit for flying.
9. L. R. S.	Observer. 50 hrs. France. Oxygen want above 12,000 ft. Very sleepy.	65′	19,000	61 ↓ 74 (21 %)	128 ↓ 138 ↓ 130	88 ↓ 80	40 ↓ 58 (45 %) ↓ 50	No change detected.	No record	+	+	+	—	Could hardly be kept awake during test. Failed at simple sum. Recommended night bombers. Did well.
12. C. H. H.	Pilot. 100 hrs. France. Breakdown after Rubella. Oxygen want at 15,000 ft. Feels very short of breath on ground. Tachycardia.	60′	15,000	96 ↓ 98 ↓ 81	128 ↓ 120 (swinging)	80 ↓ 66 ↓ 84	48 ↓ 60 (25 %) ↓ 36	100 ↓ 109 (22′) ↓ 107 (59′) ↓ 108 (69′) ↓ 114 (82′)	No record.	++	+	—	+	Sent to England. Note considerable increase in blood volume, no trace concentration. (See Chart 6.)

22. R. L. B.	Pilot. 20 hrs. France. Oxygen-want at 14,000 ft.	35′	19,000	91 ↓ 112 (23 %)	132 ↓ 138	64 ↓ 58	68 ↓ 80 (17 %)	—	Deepened and slowed. Slight periodicity towards close.	++	+	—	—	Some nervous instability. Recommended for night bombers.
27. C. A. B.	Pilot. 123 hrs. France. Oxygen-want at 9,000 ft.	28′	19,000	79 ↓ 84 (6 %)	150 ↓ 146	90 ↓ 85 ↓ 100	60 ↓ 46	—	Deepened and slowed. Some periodicity.	+	—	—	—	To England. Poor irregular pulse.
30. E. R. J.	Pilot. 100 hrs. France. Oxygen-want 'over 6,000 ft.'	47′	16,000	80 ↓ 89 (11 %)	118 ↓ 106	75 ↓ 68	43 ↓ 38	—	Deeper and faster. Some periodicity towards close.	—	+	—	—	To infantry.
32. A. H. S.	Pilot. 30 hrs. France. Oxygen-want at 15,000 ft.	49′	19,000	74 ↓ 81 (9 %)	118 ↓ 108 ↓ 118	68 ↓ 64 ↓ 68	50 ↓ 44 ↓ 50	—	Deeper and faster. Periodicity towards close.	—	+	—	—	To England. Too immature for service flying at present.
35. A. G. D.	Pilot. 273 hrs. France. Oxygen-want over 10,000 ft.	37′	19,000	64 ↓ 74 ↓ 64 (16 %)	130 ↓ 136	80 ↓ 70 ↓ 78	50 ↓ 62 ↓ 58	—	Extreme deepening and slowing. No periodicity.	—	+	—	—	Some history of C_2H_6O. To Artillery Squadron. Eventually to infantry.

5.—REPORT ON THE VALUE OF OXYGEN TO AVIATORS AT RELATIVELY LOW ALTITUDES

BY

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A. Introduction.

Early in 1917 various considerations led us, independently, to think that if flying officers were, under certain conditions, supplied with oxygen during flights beneficial results might ensue. These can be briefly summarized as follows :

(*a*) Mitigation of the fatigue always present after long flights.
(*b*) Abolition of staleness from long periods of flying at the Front.
(*c*) Increase of mental alertness.
(*d*) Increase of muscular vigour.
(*e*) Amelioration of symptoms such as giddiness, fainting, &c.

The advantages likely to accrue, if evidence in support could be secured, were obvious, but for the sake of clearness may be noted :

(1) Fewer breakdowns at the Front.
(2) The skilled pilot will be able to use his knowledge to the full.
(3) Air fights should be more successful on account of increased mental and muscular tone.
(4) The return to flying, or its continuance, will be possible by many officers whose symptoms prevent efficient air work.

Before passing to a brief survey of the physiological and other considerations that led to this investigation it should be emphasized that we have not attempted to deal with great altitude flying for two reasons : first, because the general principle that oxygen is absolutely necessary at such heights is now fully accepted ; and secondly, because if the case be proved for low altitudes and short flights it is also proved for great altitudes and long flights.

B. Physiological Considerations.

From a physiological point of view it is necessary to consider the effects of flying upon

(*a*) the cardio-respiratory system ;
(*b*) the nervous system ;
(*c*) the muscular system.

(*a*) *The Effect on the Cardio-Respiratory System.*

With increasing altitude, apart from consideration of temperature and other conditions, there is in the first place a deepening of the respiration in order to secure the oxygen necessary to maintain the bodily functions. This throws extra work upon the muscles of respiration. At the same time the heart quickens, and thus is established the beginning of a 'vicious circle'. For, an increase in the rate of heart beat means an increase in the hours of work done by the heart as shown by the following figures :

Pulse frequency, per minute.	*Ratio of systole to cardiac cycle.*	*Hours of work, per day.*
50	·31	7·5
70	·37	8·9
100	·45	10·8

This increased work entails an increased oxygen consumption, the supply of which is diminishing; thus each factor reacts unfavourably upon the other.

It has been shown experimentally that the usage of oxygen by the heart corresponds to the rate and maximal pressure of the pulse.[1]

All the devices to render the respiration and circulation efficient will therefore be called into play to meet the varying conditions, so that with prolonged strain a breakdown of the respiratory, cardiomotor and vasomotor control mechanisms is to be anticipated. The position of the pilot in the machine is such that but little help can be rendered by the pumping action of muscle movement, and even allowing for the relief given through the respiratory mechanism this is liable to become fatigued, and the circulatory mechanism must in the end suffer.

The possibility, too, of ill effects due to the washing out of carbon dioxide by the increasing depth of breathing must be borne in mind. It is a well established fact that deep breathing in itself gives rise to symptoms of marked discomfort, which frequently approach a sensation of giddiness.

(*b*) *The Effect on the Nervous System.*

In addition to the psychical strain incurred by the judgement displayed in simple straightforward flight there is the added need of quick response to changing atmospheric conditions (bumps); and in war-time flying there is superadded the strain of judgement involved in work to be done.

But this strain is not only cerebral in nature; there is the constant instreaming of impulses

(i) to the cerebellum concerned in the process of equilibration;
(ii) to the medulla oblongata in making the necessary adjustments of the respiratory and circulatory mechanisms;

[1] Barcroft and Dixon, Rohde, and other investigators (see Barcroft's *Respiratory Function of the Blood*, p. 91. Cambridge University Press).

(iii) to the spinal cord for the co-ordination of the reflex movement in the static muscular work involved ;

all this in a rarefied, and frequently in a progressively rarefying, atmosphere.

(*c*) *The Effect on the Muscular System.*

The point of prime consideration in connexion with the muscular system is that with increasing height the muscles are called upon to work with a less and less available supply of oxygen. This results in the progressive formation of lactic acid within the muscles, so that, after a flight, as Zuntz has also pointed out, the muscles of the aviator are in the condition of an athlete after a strenuous piece of work. The work of the aviator varies according as he be pilot or observer. The muscles of the pilot engaged in the direction of the machine are always in a state of semi-tension, owing to the antagonistic action of the muscles engaged in the ' static ' work performed. This state is in itself inimical to a good supply of blood, and when the disadvantage of the rarefied atmosphere is added it can be understood that a state of fatigue is easily induced without the need of a large amount of actual work being performed. The observer is usually called upon to do more actual muscular movement, and this brings about a similar state of fatigue in the muscle.

(*d*) *The Summation of Effects.*

It is not suggested that one flight, whatever be its nature, is in itself deleterious, or, except in the case of great altitudes, calls in any way for the administration of oxygen, except to those totally unfitted to fly. But, from what has been written, it will be seen that the strain of flying will tell, soon or late, upon the aviator through the cardio-respiratory, nervous or muscular mechanisms, the degree depending largely upon the individual—thus causing many of the failures in the Field.

C. Experience of Medical Boards.

That the above surmise is true is proved by experience. Medical Boards on flying officers and examination of pilots in the Field have led to the recognition of symptoms and signs, which may now be considered as directly due to prolonged air work at various altitudes. The symptoms are not so definite or simple that anything in the nature of a definite air disease syndrome can yet be claimed for them ; but their appearance and reappearance in overlapping groups is distinctly suggestive.

Thus, in the ' stale ' pilot we find definite evidence of either a breakdown in the cardio-respiratory mechanism, or in the nervous or muscular systems.

The cardio-respiratory type is recognized by an increased frequency of the pulse, which is also poor in volume and low in tension ; distress on slight exertion accompanied by a rapid rise in the pulse rate, which returns only after a long interval to its former rate. The breathing also is frequently shallow and rapid, and the extremities poor in colour and cold.

The nervous type demonstrates itself by poor muscular control over balance movements, fine tremors of hands and eyelids, greatly increased reflexes, loss of sleep, nightmares, starts, apprehensions, increasingly bad landings or giddiness in the air.

The muscular type manifests itself chiefly by tender muscles (often erroneously called rheumatism), the tone and power of which are also markedly diminished.

This last type, in its pure state, is only found among the successful pilots, and yields rapidly to rest. It is usually associated in a greater or lesser degree with the cardio-respiratory or nervous type.

This brief and incomplete *résumé* of the signs and symptoms so frequently found in the flying officer is sufficient, however, to show clearly that they correspond closely with the symptoms and signs that, it has been shown, would be expected on physiological grounds.

It is possible that objections to the use of oxygen in excess of that existing in normal atmosphere may be raised, on the grounds that it is ' drugging ' and liable to lead to temporary exaltation with subsequent depression, or to an oxygen habit.

Such objections, however, are not in any circumstances valid, and certainly not under those in which it is proposed to use the oxygen. It must be recognized that oxygen is always available under normal conditions in excess of the requirements of the body ; and it is only when we make the conditions abnormal, and repeat them day after day for long periods, and, in addition, diminish the available oxygen to a greater or less degree, that an artificial supply of this vital gas may be required.

D. Reasons for the Administration of Oxygen.

It has been shown by one of us, in conjunction with Leonard Hill, that oxygen greatly improves the condition of athletes (runners, hockey players and boxers). If administered *before* a short period of exertion, e.g. 100 yards, it may improve the time of the performance, and certainly alleviates the distress of it ; if at intervals *during* a performance, e.g. boxing, it greatly diminishes the distress caused by the previous performance, improves the form for the next round, and increases the staying power of the combatant. If administered *after* a period of long work, e.g. a three-mile race, it decreases the subsequent symptoms of fatigue—the runner will suffer little or no ' stiffness ' or other after effects. By the administration of oxygen M. F. was able to restore a delirious cross-channel swimmer to consciousness, brace him to renewed effort, enable him to surpass any previous performance and almost to achieve his end.

Briefly stated it has been shown (Hill and Flack) that the administration of oxygen :

(1) tends to keep an efficient slow pulse ;
(2) tends to keep up a good arterial pressure and delay the onset of arterial hypotension (blood pressure below normal) ;

(3) keeps off the onset of hyperpnoea or dyspnoea (distressful breathing) ;
(4) mitigates any ill effect due to excessive deep breathing.

In sport the use of oxygen is regarded as a ' dope '. In warfare it is not a question of ' doping ' but of ' efficiency '. No athlete could stand a repeated performance of hard muscular work day in day out, often several times daily, without developing ' staleness '. The aviator is in the same position, but has the extra disadvantage of the psychical strain and the performance of his task in a rarefied atmosphere. We suggest that it is not necessary for this atmosphere to be so rare as to produce immediate symptoms, then there can be no denial as to the use of oxygen, but we urge that oxygen, which has been shown to be of the value already mentioned, will from the above considerations be likely to prove useful in preventing many of the symptoms attributable to flying even at relatively low altitudes.

We have made, in support of this contention, some preliminary observations which are given below.

E. Methods of Research.

It was not found possible to carry out observations on pilots flying machines to considerable altitudes for some hours ; observations therefore were made chiefly on the B.E. 2e type of machine, making short flights of approximately half an hour at comparatively low altitudes, and flown both by pilots of experience and ones suffering from some disability. If, therefore, the results only show moderate advantage from the use of oxygen it must be remembered that short, low flights in stable machines by pilots of experience are the absolute minimum of flying effort.

The apparatus used for supplying the oxygen was the Siebe Gorman Oxygen Apparatus, similar to that originally despatched to the Expeditionary Force.

The observations taken may be divided up into cardio-respiratory and nervous. It had been intended to make the observations more comprehensive, e.g. by dynamometer tests, vital capacity measurements and alveolar air analysis, but considerations of the daily work and routine of the squadron were held to be more important, and only the smallest possible number of observations were made.

The *cardio-respiratory* observations made before and after each flight were :

(1) Pulse rate (taken twice or three times over half a minute).
(2) The ability to hold the breath (see No. 7 of this volume).
(3) Arterial Pressure, in the majority of cases by means of the small Leonard Hill sphygmomano-meter ; the accuracy of the reading being secured by one observer taking the pulse, and the other reading the instrument where any doubt existed.

The *nervous system* tests had to be limited to McDougall's dotting machine, as it had been found impossible to arrange for

wireless signalling or battery work tests on account of the unreliability of results. The McDougall machine, the use of which we owe to the suggestion of Sir Walter Fletcher, F.R.S., consists of a clockwork apparatus which passes a slip of paper through a small opening, and on the slip small circles with a dot in the centre are printed at irregular intervals and positions. The subject endeavours to spot the bull's eye of every circle as the slip of paper passes through the opening. The rate of movement of the paper is gradually increased until a breakdown is secured. The slip is then marked according to the following rules. Ten complete misses out of twenty circles constitute a complete breakdown at the particular rate at which this occurs. The rate immediately preceding this breakdown is then assessed as follows : [1]

> Plus 5 marks for a bull's eye, and minus 1 for a miss (20 circles being counted). The fastest nominal rate is 1, and for this 100 marks are given ; for rate 3, 80 marks ; rate 5, 60 marks, and so on. Thus, Lt. J. has 11 misses at rate 5 and is considered to have broken down, but at rate 6 he has only 8 misses and has secured four absolutely accurate spots = bull's eyes. His marks, therefore, would be 40 — 8 (misses) = 32 + 20 (4 bull's eyes) = 52.

Psychical.—Note was also taken of expressions of opinion volunteered by pilots, and these appear where they are considered of value in the ' remarks ' column of the data.

In regard to the *muscular system* it was felt that the flights were not of such duration or height as to warrant correct deductions being drawn.

F. Results of Observations.

Table I.

Table showing the effects of a flight upon the Cardio-Respiratory and Nervous Systems without and with the administration of Oxygen.

Name.		*Pulse.*	*B.P.*	*Breath held.* (Secs.)	*McDougall marks.*	*Remarks.*
1. Lt. K. ..	..(1) Before flight.	96/88	120	47/59	29	Flights were 20 minutes, up to 5,550 and down again.
	(2) After flight without oxygen.	108/96	95	34	24	
	(3) After flight with oxygen.	100/86	125	47/55	63	

[1] The following simple method of marking is now used : 2 marks for a bull's eye or completely within the circle, 1 mark if touching outer circle, 0 for a miss. Twenty circles are counted at each speed.

Name.		*Pulse.*	*B.P.*	*Breath held.* (Secs.)	*McDougall marks.*	*Remarks.*
2. Lt. K.	(1)	84	125	51	45	Flight (2) without oxygen was straight flight for *half* hour. (3) With oxygen approximately same sort of flight for *one* hour.
	(2)	84	95	35, 45	54	
	(3)	84	120	52, 51	47	
3. Lt. S.	(1)	74	105	61, 80	—8	In flight about half hour without oxygen. (2) Half looped, side slipped, &c. Flight (3), with oxygen, 'more strenuous'.
	(2)	96, 84	110	67, 80	32	
	(3)	75, 72	120	90	69	
4. Lt. C.	(1)	79, 84	110	73, 79	98	Both flights approximately the same. Straight up to 3,000 (without), 3,500 (with oxygen), and back.
	(2)	98, 84	120	58	124	
	(3)	96, 84	120/130	86	136	
5. Lt. P.	(1)	84	115	55	24	Both flights to 5,000 with 'stunts', more 'stunts' with oxygen.
	(2)	60	150	52, 57	—4	
	(3)	72	130	61, 68	15	
6. Sgt. C.	(1)	96	120	68	21	Straight climbing to 6,000 and back.
	(2)	84, 92	100	50, 77	7	Feeling of weirdness without oxygen; no such feeling with oxygen.
	(3)	72	125	66, 81	39	
7. Capt. R... ..	(1)	88	110 Retn.	47, 58	2	Straight flights to 3,000 and back. Subject just off sick leave.
	(2)	112/88	90, 130	69, 75	40	
	(3)	96, 92	14	85	61	
8. 2/Lt. H... ..	(1)	80	100	90	106	Contradictory result with oxygen test; arrived down with cylinder empty, and suffering partly from effects thereof.
	(2)	96	120	76	40	
	(3)	84	105	61	(not taken)	
9. 2/Lt. C.	(1)	78	105	95	25	Artillery shoot of just over an hour *without* oxygen.
	(2)	64	95	95	5	
10. 2/Lt. H. ..	(1)	96, 80	130, 120	74, 72	116	Artillery shoot of over an hour, *with* oxygen.
	(3)	74, 72	115, 120	87	148	
11. Capt. F ..	(1)	72	130	81	92	Flight of 20 min. by experienced pilot, without oxygen. Some 'stunts'.
	(2)	75	150	63	83	
12. Capt. J. ..	(1)	64, 60	—	81, 86	—	Flight of 20 min. by experienced pilot, with some 'stunts'.
	(2)	72, 68	—	62, 70	—	

Name.		*Pulse.*	*B.P.*	*Breath held.* (Secs.)	*McDougall marks.*	*Remarks.*
13. Lt. F.	(1)	88	—	56	—	Flight, 30 min. by experienced pilot,
	(2)	72	—	58	—	*without* 'stunts'.
14. Lt. F.	(1)	68	—	59	—	Flight by same pilot on another day,
	(2)	104	—	47	—	*with* 'stunts'.
15. 2/Lt. W. S. .. (Pilot)	(1)	92	—	45	—	After strenuous artillery shoot at
	(2)	140	—	25	—	1,500 feet for 30
		116	—	35	—	minutes.
		100	—	41	—	
16. Obs. S. ..	(1)	92	—	35	—	After artillery shoots without (2)
	(2)	148	—	32	—	and with (3) oxy-
		104 after 5 min.	—	44	—	gen. The oxygen result is compli-
	(3)	120	—	30	—	cated by the fact that the subject
		84	—	50	—	opened the by-
				60	—	pass, emptied his cylinder, and ar- rived with oxygen exhausted.
17. Obs. Lt. F. ..	(1)	108, 88	—	47, 57	—	An excitable sub-
	(3)	96, 80	—	61, 61	—	ject on graduated flying. Twenty minutes' flight, with 'stunts'; stated next day 'never felt so well after similar flight before'.
18. Obs. Lt. B. ..	(1)	108	—	57, 50	—	Flights to 1,500
	(2)	108, 96	—	32, 50	—	and back in 30 minutes
	(3)	88, 84	—	55, 62	—	
19. Obs. Lt. R. ..	(1)	108	—	57, 60	—	Flights to 3,000
	(2)	108	—	50, 37	—	and back in 40 min.; after oxygen
	(3)	108	—	55, 80	—	*hurried back some distance for obser- vations.*

Table II.

The effect of Administration of Oxygen after landing from Flight.

	On landing			*After one minute's oxygen.*		
Subject.	*Pulse.*	*B.P.*	*Breath held.* (Secs.)	*Pulse.*	*B.P.*	*Breath held.* (Secs.)
E. L. (Observer)	108	—	55	72	—	74
R. McD. „	80	—	74	72	—	121
B. „	120	85	18, 20 (after exercise 16)	87	110	24 (after exercise)

Discussion of results.—It is not pretended that these observations on flights at comparatively low altitudes and generally of short duration are altogether conclusive. Certain facts, however, appear to emerge. In regard to the cardio-respiratory system, the results appear to be analogous to those already observed by Hill and Flack as the result of exercise. In general, the pulse is accelerated, the arterial pressure raised, and if the effort be prolonged or cause strain a hypotension supervenes. It is, perhaps, well not to pay too much attention to the pulse rate before a flight owing to the initial excitement, especially in subjects of a nervous temperament. A short flight without oxygen appears to have little effect upon an experienced normal pilot. If he has stunted at all he may have acceleration of the pulse, an increase in arterial pressure, a diminished power to hold the breath, and some nervous exhaustion as shown by the McDougall machine.

To pilots to whom the strain of flying is greater (e. g. Table 1, Nos. 1, 2, 6, 7) there results an arterial hypotension analogous to that of prolonged exercise. The administration of oxygen has a beneficial effect, the subject, even when of an excitable nature, landing with a slow, full pulse, a normal or raised arterial pressure, an increased power to hold the breath.

The abolition of the onset of the hypotension of arterial pressure is of great significance (Table I, Nos. 1, 2, 6, 7).

The results of the observations 9 and 10, Table I, are suggestive ; 2/Lt. C. and 2/Lt. H. make approximately the same performance (an artillery shoot of one hour's duration). Lt. C. arrives back with some signs of circulatory and nervous fatigue. Lt. H., although slightly longer in the air, arriving back in good condition with no signs of fatigue, his only complaint being of the smell of the mask used.

The results with the dotting machine indicate an all-round improvement with the administration of oxygen. Of nine cases, six deteriorate after the flight without oxygen and three improve ; after the flight with oxygen all make improved performances, and in the cases where there was previously a deterioration surpass the previous performances.

The contradictory results, obtained when the oxygen supply fails, point to the necessity of the employment of a thoroughly well tested and reliable apparatus.

The beneficial results of oxygen administered after a flight are well seen in two of the three instances given in Table 2. Observer B. is a particularly interesting case. After a flight of twenty miles at 1,000 feet he arrived with a rapid pulse, an arterial pressure of 85, and a very limited power to hold the breath (only 16 seconds after the exercise of touching the toes four times). After the administration of oxygen for one minute, the pulse was slower, the blood pressure and power to hold the breath increased. This officer has already fainted in the air three times in six hours' flying experience. Similar results, especially in the improvement of the rate of pulse, have been obtained by the administration of oxygen to pilots home from overseas with cardio-vascular debility after long periods of service.

No observations have been made as to the effect of administration of oxygen after flight upon the performance with the dotting machine. Two instances may be quoted in which the administration of oxygen for one minute to mentally fatigued persons improved the performance 30–40 per cent.—in one case raising the percentage of hits from 65 to 97·5, with the machine going at a medium rate.

G. Discussion as to the best methods of Administration of Oxygen.

The method of continuous administration has been considered best for aviators owing to the fact that the atmosphere is rarefied. It is the method which we have employed in our experiments. The method necessitates the continuous wearing of a mask and requires a relatively large supply of available oxygen. When only limited supplies of oxygen are available we would suggest, however, that there is much to be said for an intermittent supply of oxygen when in the air. Several pilots also have stated that they could not fight with a mask on the face and with a tube in their way. As a pilot of experience said : ' Would it not be better to have a good dose when the Hun was coming, then put it aside and go for him and take another dose when he was finished with.'

When oxygen is not carried for any reason we suggest that it would be a good practice to administer oxygen for a few minutes to all airmen after heavy flights at any altitudes, e. g. bombing raids, &c. We support this contention from the effects obtained by administration to three-mile runners. In our opinion such administration would, as with the long-distance runner, abolish much of the effects of fatigue and appreciably increase the length of service in the field.

H. Conclusions.

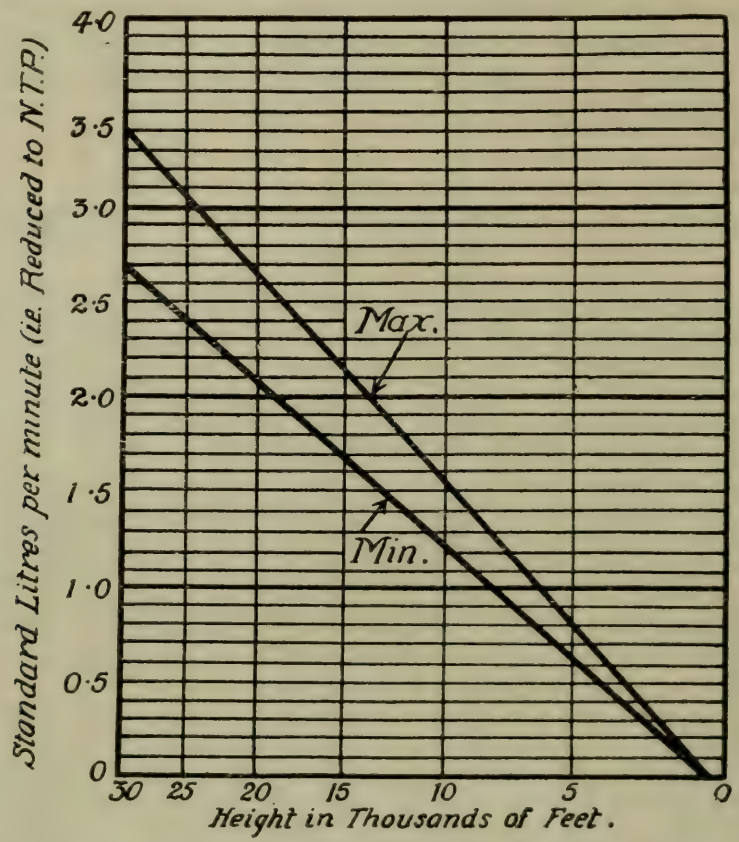
It is provisionally concluded that :

(1) In flights at low altitudes there is a certain degree of cardio-respiratory and nervous fatigue, varying with the nature of the flight and with the individual ;
(2) The administration of oxygen abolishes this fatigue in short flights, and delays its onset in longer flights ;
(3) The administration of oxygen after a flight improves the cardio-respiratory condition of the subject.

Addendum.

After the date of the first circulation of this report, oxygen was used overseas in increasing amount on high-flying machines. It was supplied from light cylinders carried beneath the lower planes, its delivery being regulated by an apparatus designed by Professor Dreyer (Dreyer Apparatus Mark I or Mark II). Administration was continuous by means of tubing and a special mask. The results

were satisfactory, the following graph showing the amount of oxygen given at various altitudes.



6.—THE SELECTION OF CANDIDATES FOR FLYING

REPORT ON THE EXAMINATION OF A SERIES OF SUCCESSFUL PILOTS AND UNSUCCESSFUL PUPILS FROM THE POINT OF VIEW OF THEIR CARDIO-VASCULAR AND NEURO-MUSCULAR SYSTEMS

BY

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A. Introduction.

The object of this examination was in part to see what type of subject, if any, makes the most efficient pilot, and also in part to ascertain if the standards adopted by the Commissions Board for the admission of pilots were too strict, especially from the point of view of cardio-vascular and nervous debility.

At the same time it was hoped that the examination would yield information as to the degree of importance of some of the tests employed by the Commissions Board.

The routine procedure of the examination was as follows :

(*a*) Age.

(*b*) *Flying History.* Details of admission to R.F.C., number of hours' flying, average height, maximum height.

(*c*) *Medical History.* History of concussion, faints, serious illnesses, chilblains, &c.

(*d*) Pulse conditions at rest, with the arm in the horizontal and in the vertical positions.

(*e*) Systolic and diastolic sphygmomanometer readings of blood pressure, with the arm in horizontal (heart level) and vertical positions.

(*f*) Condition of jugular veins as regards engorgement in the recumbent position, without and with pressure on the abdomen.

(*g*) Condition of abdominal wall as regards firmness, and of stomach as regards splashing.

(*h*) Reflexes, tremors, and equilibration.

(*i*) Effect of exertion (raising the body on a chair 5 times in approximately 15 seconds) on the pulse rate ; also the rapidity of return of pulse to the rest rate after such exertion.[1]

[1] No tests for the respiratory system were included, since these were already being investigated. (See 'Tests for Flying Efficiency', &c., No. 7 of this volume.)

B. Material.

Flying officers engaged in various capacities have been examined ; these may be grouped as follows :

(*a*) O.C.'s, Flight Commanders, and Instructors of Flying Schools	29
(*b*) Test Pilots	17
(*c*) Home Defence Pilots	30

These may be conveniently grouped into those at present employed on low-flying machines (15) and those on scouts (15).

The opinion of the O.C. was taken as to the capabilities of each officer examined. In addition we examined 17 good pupils and 8 unsuccessful pupils, as chosen by the O.C.

[Later (June 1918) one of us (A. P. B.) examined the records of a series of successful and unsuccessful pupils, particularly from the point of view of the neuro-muscular system (see Section E).]

C. Results.

(*a*) *Age.*

The average ages of the series may be tabulated as follows :

Pilots.	*Years.*
Flying Schools	26·1
Test Pilots	24·7
Home Defence :	
Low Flying	22·1
Scouts	23·8

Observations.—Scrutiny of the capabilities of these pilots has demonstrated that the man of 20–27 years is more likely to make an efficient flying officer than youths of 18–20.

Since flying exposes the human organism to a greater strain than it has probably ever been exposed to before, it appears to us that the subjects should as far as possible be fully grown, so that the whole of the resources of the organism be available to combat the great strain involved. It is hardly to be expected that growing youths can stand up to this strain as well as men of 20–27. For this reason we would suggest, as our observations indicate, that officers and men up to the age of 30 be encouraged to become pilots. From a medical point of view the man of 22–27 is more likely to make good in the air ; conversely, many youths of 18 now passed for pilots would really be more efficient in the infantry. By this it is not meant to suggest that no candidates of 18 should be accepted. It is necessary to bear in mind that the cardio-vascular and nervous systems at this age are frequently unstable, owing to immaturity, and that these youthful candidates have often been subjected to little or no nervous stress. How they will meet such stress can only be determined by the test of actual flying. In this test a fairly high percentage are now found wanting, at considerable cost in time and money to the country. For these reasons alone it appears advisable in recruiting for service in the air to draw more generally from a class of men in which some degree of natural selection has been at work, e. g. a period of service in the trenches.

(*b*) *Flying History.*

Practically all the candidates had been admitted without special medical examination at the Air Board, and therefore represent the survival of the fittest.

The average number of hours flown and the average height of flying of the three classes of pilots is as follows :

	No. of hours flying.	*Average height.*
Flying schools	574·6	8,890
Test pilots	418·4	7,770
Home defence—		
On low-flying machines ..	271·1	8,630
On scout machines ..	362·6	10,730

(*c*) *Medical History.*

The outstanding feature of the medical history was the number of pilots who had suffered from concussion at some period of their career (approximately 40 per cent.). In some cases it was severe ; for example, Lt. E. G., M.C., while in the infantry was buried twice in one day, was unconscious for about half a day, in hospital for three months, with speech affected for three weeks. Since admission to the R.F.C. he has done eight months flying in France, is now engaged in Home Defence night flying, feels fit, and is described by his O.C. as a good steady pilot.

Other pilots gave a history of periods of unconsciousness up to seven days. One pilot who had sustained a fractured base, and various other injuries, and was unconscious five days, is now doing useful work as instructor. It would appear that in the absence of any signs of nervous instability, except in recent cases, a history of concussion or unconsciousness due to head injury is a point in the subject's favour as showing the ability to withstand these shocks to the nervous system, perhaps also as indicating in many cases the enthusiasm and recklessness characteristic of the flying temperament.

A certain number of subjects examined had suffered from bad headaches in childhood, but had experienced no recurrence since flying.

The fact that some pilots experienced considerable headache when flying at high altitudes, but not when flying at lower altitudes, e. g. on artillery observation, emphasizes the fact that it would be well if pilots could be graded for the type of work for which they are most suitable. A pilot, for example, who suffers from headaches at altitudes is more likely to perform a lengthy period of good work at 8,000 than at 15,000 feet.

(*d*) *Physique.*

Practically all the subjects were of good physique. It cannot be said that any type of physique is especially characteristic of the flying man. There were examined the sturdy stock type, the tall lean type, &c.

It would appear from this series that a good physique is of the greatest importance, especially in the high flyer.

Physique, however, is not the whole story, since flying is essentially a question of temperament.

It must be confessed that in our opinion the ' flying temperament ' largely baffles assessment at present. Thus we examined a pupil who, from the medical point of view, should have made an efficient flying officer. We learnt that he had practically to be driven into the machine. We also met several other cases of officers who exhibited marked disinclination to fly, but in whom no apparent medical symptoms were manifest to account for this.

(e) *Circulatory System.*

Pulse rate.—The pulse rate at rest and after exercise, together with the time taken to return to rest rate, is given in the following table :

	Pulse rate at rest.	*Pulse rate after exercise.*	*Increase.*	*Time taken to return to rest rate.* (Secs.)
Flying school	87	114	27	20
Test pilots	84	109	25	25
Home defence—				
Low	101	126	25	16
High	85	110	25	18

Blood pressure.—The average systolic and diastolic pressures in the series are given below. These were taken with the arm at heart level and also with the arm raised through an angle of 120°. Both the systolic and diastolic pressures were taken by the auscultatory method, the systolic pressure being read preferably at the point of reappearance of the pulse (on coming down after previous obliteration by the armlet pressure).

	Arm at heart level.			*Arm raised through an angle of 120°.*		
	Systolic pressure.	*Diastolic pressure.*	*Pulse pressure.*	*Systolic pressure.*	*Diastolic pressure.*	*Pulse pressure.*
Flying school ..	120·6	89·3	31·3	100·2	70·2	30·0
Test pilots ..	121·4	82·8	38·6	103·2	63·5	39·7
Home defence—						
Low	119·1	77·7	41·4	98·5	58·0	40·5
High	122·5	74·9	47·6	102·9	52·6	50·3

Observations.—From a consideration of our observations on the cardio-vascular system it is possible to draw the following conclusions :

(*a*) That the observation of increase of pulse rate after exercise, and the time it takes to return to the rest rate, is a useful method of testing the functional efficiency of the cardio-vascular system. With the method of exercise adopted by us an increased rate of over 25 and a return period of over 30 seconds are points calling for careful consideration of the candidate's cardio-vascular system. It is to be observed that a rest rate above normal, especially if influenced by respiration, is not regarded as necessarily throwing doubt upon the functional efficiency of the cardio-vascular system. A pulse of 60 to 72 little raised by exercise (10 beats per minute), returning to

normal in 10 seconds, is in our opinion a good sign, generally associated with excellent physique and good stability of the nervous system.

(*b*) From a consideration of the blood pressure records, the conclusion is forced upon us that stress of service or high flying almost invariably produces a low diastolic pressure and a big pulse pressure, due probably to lessening of vaso-constrictor tone of the arterioles.

A diastolic pressure below 70 mm. Hg, combined with a pulse pressure greater than 50 mm. Hg, is strong evidence that a cardio-vascular system is unsuited for air work. In showing how this rule would have worked out in actual practice among the various groups examined, the following table will be of value :

	No. examined.	*No. of P.P. over 50.*	*% P.P. over 50.*	*No. of D.P. below 70.*	*% D.P. below 70.*	*No. of men with P P 50+ and D.P. 70 —.*	*% with P.P. 50+ and D.P. 70 —.*
Flying school instructors ..	29	0	0	0	0	0	0
Test pilots ..	17	4	23·4	3	17·6	2	11·7
Home defence—							
Low	15	3	20·0	1	6·6	1	6·6
Scout	15	6	40·0	3	20·0	3 [1]	20·0
Pupils under instruction—							
Successful ..	17	4	23·4	5	29·4	0	0
Failures.. ..	8	5	62·5	4	50·0	4	50·0

It should be borne in mind that in the case of test pilots recent nerve strain was common, and in the Home Defence Squadrons the pilots were being subjected to fairly constant stress, and there is every reason to believe that with a period of rest these would conform to the requisite standard. The instructors at flying schools and the successful pupils satisfied this test without exception, while four of the eight unsuccessful pupils would have been rejected on the score of cardio-vascular deficiency. The other unsuccessful pupils, it should be observed, were of the nature of the ' temperamentally unfit '. It is not suggested that this test should be applied rigidly without consideration of other points in the candidate's condition.

Indeed, in all subjects failing in this test, and particularly borderline cases, the other points (soft abdominal wall, splashing stomach, engorged jugular veins, reflexes (brisk or exaggerated), tremors, poor balance, local signs) should receive due consideration. In this series it was found that failure to comply with this blood pressure test was almost invariably associated with two or more of these abnormal physical signs. Indeed, all these signs are presented by the advanced cardio-vascular defective.

The ideal pulse for a flying officer has a small range between systolic and diastolic pressures (20–30), with a rest rate increased

[1] One of these has since been killed; he was passed by the Commissions Board as observer only, unfit as pilot. Another has been taken off flying for a time (Jan. 1918).

at most 20–25 by exercise and returning to the rest rate in 10–15 seconds.

Engorgement of jugular veins.—In regard to the condition of the jugular veins, sixteen pilots exhibited slight engorgement. It is thought that engorgement of the jugular veins, with the subject in the recumbent posture, points to a heightened venous pressure due to lax arterioles. When pressure on the abdominal wall greatly increases the engorgement, it is looked upon as a sign of ' splanchnic pooling '. This engorgement of jugular veins is almost invariably to be found in severe cardio-vascular deficiency. In those of the series in which it was observed the condition was marked in one case only, an advanced cardio-vascular and nervous defective, still under instruction, who had been categorized by the O.C. as unfit for flying.

Local signs of deficient circulation.—Chilblains and blue hands were met with in seven cases among the pilots examined. In regard to signs of deficient circulation, one point is perhaps worthy of comment. It was ascertained that pilots, whose duty it was to take the air at any moment, frequently had to ascend to great heights in boots and puttees, since these cannot be quickly discarded. Several pilots stated that when flying in boots and puttees they suffered from numb feet, although they did not so suffer when otherwise clad. This statement may of course be somewhat prejudiced owing to the pilot's predilection for shoes and stockings, but, from a medical point of view, puttees are an exceedingly undesirable form of leg wear for any pilot in the air, owing to the fact that the limb volume may be greatly restricted, and the effective circulation of blood thereby hampered.

(*f*) *Muscular Tone.*

Condition of abdominal wall.—The condition of the wall, as judged by palpation, was found to be variable. It was graded as firm, fair, or soft, as follows :

	Firm.	*Fair.*	*Soft.*
Flying school	10	7	12
Test pilots	10	4	3
Home defence—			
Low	11	9	5
Scout	3	6	6

Stomach splash.—This was observed in eleven subjects.

Observations.—The condition of the abdominal wall as regards firmness, and of the stomach as regards absence or presence of splash, affords some information as to the general muscular tone. It is noticeable that the tone of the abdominal wall was less satisfactory among trained pilots than among successful pupils under instruction (see Section D). This indicates that one of the first signs of flying stress is to be found in the loss of tone of the abdominal muscles. This was confirmed later by a special test with a U tube manometer (see ' Tests for Flying Efficiency ' in this volume). A soft ' doughy ' abdominal wall, with a splashing stomach, in addition to denoting lack of tone by allowing the abdominal pressure to fall, aggravates any tendency to splanchnic flooding. Taken in conjunc-

tion with the condition of the other systems, the presence of a soft abdominal wall and splashing stomach is to be viewed with suspicion.

(g) *Nervous System.*

Reflexes.—These were tested especially as regards knee jerks, and in most cases as regards also supinator and triceps jerks. The results of the knee-jerk test were graded as 'sluggish', 'normal', 'brisk', and 'exaggerated', and may be summarized as follows:

	Sluggish.	*Normal.*	*Brisk.*	*Exaggerated.*
Flying school	4	11	13	1
Test pilots	5	1	11	0
Home defence—				
Low	5	5	5	0
High	5	3	6	1

Tremors.—The chief test employed was to examine the fingers, semi-flexed and separated, with the eyes shut and the arms outstretched in front of the subject. The condition of the eyelids, when the eyes were shut, was also generally observed. The degree of tremor was graded as 'absent', 'slight', or 'marked'.

	Absent.	*Slight.*	*Marked*
Flying school	14	9	4
Test pilots	11	6	0
Home defence—			
Low	9	6	0
High	12	2	1

Observations.—Brisk reflexes were not uncommonly met with in pilots doing good work. The knee jerks were markedly exaggerated in three cases: one an instructor who had recently done considerable high flying in France, one a scout Home Defence pilot, a fine stunter, who was suffering from a bad cold and feeling unfit, and one an unsuccessful pilot under instruction presenting signs of considerable cardio-vascular debility and nervous instability. In regard to tremors, these are absent or slight in the good pilot. Those present in the officers of the flying school might be attributed in two cases to periods of strenuous service overseas from which they had just returned; the two others were possibly associated with excessive smoking. This series demonstrates the test as a valuable one, an exaggerated tremor being undoubtedly a disqualification for flying.

As regards equilibration, five successful pilots and three unsuccessful pupils exhibited poor equilibration. Four of the former were high flying scouts, one of whom had a cold and did not feel fit. Slight sway was observed in a small proportion of all groups. The balance of the great majority was excellent. We find therefore that a high standard of equilibration is to be required in pilot candidates.

The exact significance of a slight degree of 'neuro-muscular instability' baffles assessment at present. Thus a subject exhibiting a certain degree of nervous instability turns out in one case to be an excellent 'stunt' pilot, in another case to be useless.

From the point of view of stress on the nervous system we make the following suggestions in regard to the training of pupils under instruction. It appears to us that the practice of testing the

'nerves' of a pupil by 'stunt' performances during his first instruction flights casts an unnecessary strain upon the nervous system. It is possible that the disinclination to fly which sometimes develops during training may be ascribed to such performances. Also in regard to the first solo flight it is better practice, as we understand is often done by instructors, to take the pupil for a preliminary trip, and, if this be satisfactory, to allow him, in the enthusiasm of the moment, to venture out on his first solo rather than to inform him overnight of the intended solo, which he then may anxiously await in a state of nervous trepidation. We would point out also that it would be well if only pilots in whom there is no suspicion of nervous instability be employed as instructors, otherwise they tend to affect the 'nerves' of the instructed, or to instruct inefficiently. It is not fair to the pupil that his instructor should be a pilot suffering from 'nerves' in any way.

D. Observations in Pilots under Instruction.

Although reference has been made, from time to time, as to the results obtained from pupils under instruction, the chief points observed are given in detail here. In addition to the successful pilots, twenty-five pupils under instruction were examined. The same observations were made as in the first series. After the examination was completed, the O.C. divided the pupils into those who were promising to make good pilots and those who were failures.

The result of the examination of these, as regards pulse rate (rest after exercise and return), blood pressure (systolic, diastolic pulse), is summarized below:

	Rest rate per min.	*Rate after exercise.*	*Time to return in secs.*	*Systolic pressure in mm. Hg.*	*Diastolic pressure in mm. Hg.*	*Pulse pressure.*	*Systolic pressure (arm raised).*	*Diastolic pressure (arm raised).*	*Pulse pressure*
Successful	85	109	21	115·7	74·1	41·6	97·1	53·9	43·2
Failures	85	121	23	122·1	68·4	53·7	103·7	51·1	52·6

The result of the examination for the other points observed is as follows:

	Abdominal wall.			*Stomach splash.*	*Jugular veins.*	*Reflexes.*				*Tremor.*			*Equib.*			*Local signs.*	*Concussion.*
	Firm.	*Fair.*	*Soft.*			*S.*	*N.*	*B*	*Ex.*	*Ab.*	*Sl.*	*Mk.*	*G.*	*M.*	*P.*		
Successful (17)	14	3	0	2	4	3	6	8	0	9	7	1	13	4	0	1	5
Failures (8)	2	4	2	2	1[1]	1	3	3	1	1	3	4	2	3	3	3	4

Note.—*S.* = Sluggish. *N.* = Normal. *B.* = Brisk. *Ex.* = Exaggerated. *Ab.* = Absent. *Sl.* = Slight. *Mk.* = Marked. *G.* = Good. *M.* = Moderate. *P.* = Poor

[1] Much engorged.

In our opinion all the successful pupils would have been admitted. In regard to the unsuccessful pupils, a number would have failed, but what may be termed the ' temperamentally unfit ' would probably have been passed at the time when this investigation was made.

E. Analysis of Records of Successful and Unsuccessful Pilots and Pupils.

To amplify the small number of observations made above, one of us (A.P.B.) analysed the records of a series of candidates especially from the point of view of the association of signs of neuro-muscular instability with failure to train as a pilot.

With the exception of the records of Group II, which have already been dealt with, the observations were obtained either from the original medical examination sheet or from medical reports made subsequently at the Invaliding Board. A pupil has been considered successful if he has completed his training and obtained his wings; an unsuccessful pupil for the purpose of this report is one who has been declared by the Invaliding Board permanently unfit for air work, or who has crashed fatally.

The cases whose records have been investigated and compared are included in four groups :

Group I. 1,000 accepted candidates who ultimately became successful pilots. Records taken from original medical examination form, on admission.

Group II. 93 successful pilots and pupils. Of these 76 had qualified and had considerable flying experience, and 17 were successful pupils about to obtain their wings. Records taken on aerodromes.

Group III. 200 accepted candidates who eventually failed to qualify or crashed during training with fatal results. Records taken on admission.

Group IV. 40 pupils under instruction who failed in their training, and were declared permanently unfit by the Invaliding Board. Crashes are not included in this group. Records taken by the Invaliding Board.

The following tests were applied to determine respectively the condition in regard to equilibration, tremor, and reflexes :

(i) Standing for fifteen seconds on one foot with the eyes closed, hands to sides ; the raised leg flexed at knee, with foot behind. Only cases showing marked unsteadiness or inability in several attempts to accomplish this test were noted.

(ii) Standing with hands outstretched, fingers separated and slightly flexed. Cases of pronounced tremor only were noted.

(iii) Raising a board on which a tuning-fork was balanced from a table to the level of the shoulder, with the arm extended, and lowering the board to the table again without upsetting the fork.

(iv) Knee jerk. Only cases were noted in which the reflex was definitely exaggerated, mere briskness of the response was disregarded.

The table below shows the percentage of the cases examined which have unfavourable reactions to one or more of the tests.

	Group I.	*Group II.*	*Group III.*	*Group IV.*
	1,000 Successes Records on Admission. Per cent.	*93 Successes Records after Training. Per cent.*	*200 Failures Records on Admission. Per cent.*	*40 Failures Records Invaliding Board. Per cent.*
Poor equilibration alone	1·6	5·3	8·0	5·0
Marked tremor alone	2·0	6·4	5·0	20·0
Exaggerated reflexes alone	3·9	2·1	2·4	2·5
Combination of two of the signs above mentioned	0·5	0·0	3·0	40·0
Combination of three of the signs above mentioned	0·1	0·0	1·0	17·5
Total percentage presenting signs	8·1	13·8	19·4	85·0

It is shown by this table—

(I) That the percentage of cases which gave a bad reaction to the tests are very much greater among the failures —prospective or actual—than among the successes.

(II) That these signs of instability are more obvious in men who have actually flown, whether they have graduated successfully or have failed, and that therefore they may be regarded as signs of the result of ' strain ' of flying. The results in Group II are worse than in Group I, and similarly those in Group IV are much worse than those in Group III.

(III) A comparison of the percentages presenting the various signs or combinations of signs in Groups I and III would appear to show that the presence of poor equilibration or marked tremor seriously diminish a candidate's chances of gaining his wings. Indeed, it is probable that ' fair ' equilibration and a moderate degree of tremor are indicative of likely failure to qualify as pilot. Exaggerated reflexes, on the other hand, appear to be unassociated with any abnormal tendency to failure. Candidates who present these pronounced signs in combination are so rarely found to be fit in other respects that the number accepted is extremely small. Such figures as are available are, however, highly suggestive of the inadvisability of ever accepting these candidates. This view receives additional support from a consideration of the figures in Group IV, which show the condition as regards these points of 40 unsuccessful pupils on their appearance before the Invaliding Board after failure had occurred.

It is interesting to note that exaggerated reflexes and pronounced tremor occurred in 22·5 per cent. of these cases, exaggerated reflexes and poor equilibration in 2·5 per cent., pronounced tremor and poor equilibration in 15 per cent., and all three signs in 17·5 per cent.

F. Conclusions.

In regard to the standards of the Commissions Board, it may be taken that the would-be pilot is subject to a certain amount of strain by the medical examination to which he is subjected, and that due allowance should be made for this. But it is to be remembered that the strain of this examination is by no means so great as that to which he will be subjected, first, when he begins his solo flying, and later, when called upon to engage in combatant service in the air.

At such times the cardio-vascular and nervous system are subjected to enormous strain and breakdown is of comparatively frequent occurrence. It is of the highest importance, therefore, that no subject with signs of cardio-vascular or nervous instability be admitted at the preliminary examination, the chances being that such a subject will in all likelihood break down during the first period of stress, the stress of training, or if not, will be rendered in such a state that collapse under stress of service is almost inevitable.

It is imperative that the standard adopted allow a considerable margin (1) for the coming stress of training, and (2) for the prospective stress of service.

In regard to the selection of candidates for flying it may be concluded :

(I) That the pulse rate should not be unduly increased by exercise and should quickly return to normal. To ensure this the test here employed (lifting and lowering the body weight to and from a chair 5 times in 15 seconds) is of value, being capable of quick application and affording satisfactory results.

(II) The measurement of the systolic and diastolic pressures is important. A diastolic pressure below 70 mm. Hg. combined with a pulse pressure greater than 50 mm. Hg. is strong evidence that a cardio-vascular system is unsuitable for air work.

(III) A firm toned abdominal wall is necessary in a candidate. Lack of tone of the abdominal muscles is one of the first signs of flying stress.

(IV) As regards the neuro-muscular system it may be concluded that

(*a*) Exaggerated reflexes alone appear to have no prognostic value, and their presence may be disregarded ;

(*b*) Marked tremor and poor equilibration may be taken as signs of poor neuro-muscular stability. The case of a candidate who shows one of these signs only should receive very careful consideration. It is justifiable

to assume that his chances of making a successful pilot are much less than normal;

(*c*) A candidate who shows both marked tremor and poor equilibration is probably unfitted for air work and should be rejected forthwith, unless his condition can be ascribed to some temporary indisposition. The chances are not in favour of his being able to train successfully, and even if this were accomplished, his career as a useful pilot would probably be very short. Under such circumstances the expense to the country, the danger to life involved, and the waste of time to instructors are considerations that render the acceptance of such a candidate unjustifiable;

7.—TESTS FOR FLYING EFFICIENCY AND FLYING STRAIN[1]

BY

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A. INTRODUCTION.

MODERN flying, by its complex and nerve-trying evolutions in a rarefied atmosphere, imposes a great strain upon the aviator, especially at high altitudes. In consequence breakdown is frequent, the patients being found to suffer from gradual loss of power to fly high, associated in addition to frequent psychological manifestations in varying degrees with particular symptoms of cardiac and nervous derangement, like those of quickened pulse rate, increased pulse pressure, splashing stomach, nervous tremor of fingers and eyelids, or poor power of balance.

It is therefore a point of the greatest medical importance that some simple test or tests should be devised by which an aviator, in the incipient stages of breakdown, can be singled out so that the condition may be checked and remedied. It is believed that the simple tests described below, taken in conjunction with other points in the clinical examination, will serve (*a*) to aid in the efficient selection of candidates for flying service, and (*b*) to demonstrate in serving officers the need for rest, or at least for lessened work, at a stage when the pilot himself frequently does not feel justified in complaining for fear of being regarded as a shirker.

Various considerations led the writer to the conclusion that the first breakdown in the bodily systems is probably in connexion with the respiratory mechanism, which, becoming defective in working, reacts, owing to the 'anoxaemia' induced by inefficient respiratory ventilation, upon the cardio-vascular and nervous systems, thereby bringing out secondary signs of cardio-vascular and nervous debility.

Such a view is quite reasonable when it is remembered that it is on the respiratory system that the strain first falls in rarefied atmospheres.

In the first place, by the correlation of simple with more elaborate methods, an attempt was made in April 1917 to devise tests which might exclude from the flying services candidates who were likely to suffer from discomfort in the air, headache, giddiness, fainting, dizziness, nausea, vomiting, pressure in the head, blood rushing to the temples, palpitation of the heart, &c., symptoms which might be due to lack of proper oxygenation of the blood and were likely to render them unfit as flying officers.

[1] This report represents a combination of Report No. 1 (I) and Report No. 3 originally published. Report No. 1 was first circulated type-written in July 1917 and printed February 1918; Report No. 3 was first printed April 1918.

B. Methods of Research.

From a number of pilots a sample of blood was taken, the haemoglobin content, the corpuscular count, and the total oxygen capacity after submission to rarefied atmosphere, determined.

The first two points were barren of results ; of the third it is not fair to speak, since the conditions under which the work was done were not suitable for so delicate a technique, and for this reason this method of research was discarded after about ten examinations. The research then proceeded by the taking of

(*a*) the tidal air ;
(*b*) the vital capacity ;
(*c*) the composition of the alveolar air after full expiration and full inspiration ;
(*d*) the composition of the alveolar air after full expiration and inspiration and holding the breath as long as possible, the length of time being noted ;
(*e*) by getting the subject to breathe in and out of a bag (described earlier), the carbon dioxide being absorbed by a cartridge containing coke coated with caustic soda.

It is not proposed to give here the results of all the analyses of the alveolar airs. The oxygen content of the alveolar air after holding the breath gave an idea of the amount of diminution of oxygen which can take place in the alveoli before discomfort is induced. This was found to vary from 13·5 to 10 per cent. according to the individual. The idea underlying the test of holding the breath is that, during the holding of the breath, the oxygen content of the air in the lung becomes less and less, so that the subject is, so to speak, at the time ascending to a rarer atmosphere and therefore the degree of rarefaction which can be tolerated without discomfort gives an indication of the height to which an individual can ascend without discomfort. It was argued that a man who can hold his breath long, until the alveolar oxygen content is low, should make a good altitude flyer ; a man who can hold his breath but a short time should make but a poor high flyer, or a flyer with poor resolution. This test therefore has been carefully correlated with the bag experiment already described, to which the objections as to the accumulation of carbon dioxide and the embarrassment of the circulation do not apply.

From the point of view of simple tests, therefore, attention was in the first place directed to the vital capacity, and the power to hold the breath after full expiration and inspiration.

As a preliminary measure, figures were obtained in the summer of 1917 from 40 normally successful pilots who were admitted to the flying services without any special medical examination. They were officers of great experience and with three exceptions had flown for 200 hours or more. Furthermore, the pilots in question were selected for examination by the executive authorities, on account of the merit of their flying services. As the result of these preliminary observations, it was found that the minimum vital capacity of any successful pilot was 3,400 c.c. and the minimum time for holding

the breath 46 seconds, this in the case of a ' stale ' pilot direct from hard work overseas. The average vital capacity was approximately 3,950 c.c.[1] and the average time for holding the breath a fraction over 66 seconds. In every case what might be termed a normal answer, such as ' I wanted more breath ', ' I felt like bursting ', was stated as the reason for giving up holding the breath. In marked contrast were the results obtained from a number of officers exhibiting symptoms possibly attributable to lack of oxygen early in their career. A number of results are set out in Table I.

It will be seen at a glance that all the above failed to reach the standard achieved by the successful flying officer.

The remarks of the officer after holding the breath are recorded in the column for the purpose and afford a good idea of what may be deemed an ' abnormal ' answer. In many cases the act of holding the breath reproduced the symptoms from which they suffered in the air. In view of their short flying experience, it appears probable that these officers would have been eliminated at their entrance medical examination on the tests which they now failed to pass.

On the basis of these results, it was therefore suggested in July 1917 that the following standards be provisionally adopted by the Commissions Board :

(1) Rejection of all candidates with vital capacity below 3,000 c.c.

(2) Rejection for high flying of all candidates who have a vital capacity below 3,400 c.c. and who hold their breath below 45 seconds (in three times), and *give an answer indicative of ' oxygen want '*.

(3) Rejection or deferring of candidates who have a larger vital capacity (over 3,400) but only hold breath 45 seconds or under.

Owing to the difficulty of obtaining suitable meters, the vital capacity measurement was not used to any great extent ; but the breath-holding test was adopted for routine purposes. The results have undoubtedly been beneficial since during the past two years it has become progressively rarer for officers to complain of untoward symptoms during their first few hours in the air.[2]

A valuable variant of the breath-holding test was found to be that of holding the breath after regulated exercise, e. g. raising the body 5 times on a chair in 15 seconds.

[1] According to the calculations of Professor Dreyer this figure indicates a certain amount of fatigue (about 10 per cent.) due to stress of service. [See *Lancet*, Aug. 9, 1919, p. 227.]

[2] Lieut.-Colonel Bowdler, O.B.E., has supplied me with the following figures showing the percentage of pilots of various breath-holding capacities breaking down within one year of acceptance by the Aviation Candidates Medical Board. No results of crashes are included in these figures.

Breath-holding time in seconds.	*Number of Pilots.*	*Permanently or Temporarily unfit for long periods during first year.*	
		No.	*Per cent.*
40–50	131	17	13·0
51–60	273	28	10·2
61–70	208	16	7·7
Over 70	263	18	6·8

TABLE I

Name.	*Vital capacity in c.c.*	*Time breath held.* (secs.)	*Remarks after holding breath.*	*No. of hours flying.*	*Max. height in ft.*	*Average height in ft.*	*Remarks.*
2/Lt. E.	2,100 (Max. 2,400)	25	Made him fuzzy.	10½ (dual)	6,000	1,000	Giddiness and blurring of vision when flying at 6,000. Bag method broke down at 6,000 ft.
2/Lt. S.	4,100	42 (Max.)	All out, dashed uncomfortable.	33	9,500	2,000	Nausea above 7,000 ft. (only three times ever above this, very bad at 9,500). Bag experiment, 9,000 ft.
2/Lt. A. L.	4,200	31	—	4 (as observer)	6,000	2,500	Great difficulty in breathing, and dizziness at 5,000 after four hours' flying.
2/Lt. Bo.	3,800	19	Head inclined to swim.	6 (dual)	6,000 (once)	—	Has fainted three times below 5,000 ft. With bag method nearly fainted at 3,000 ft.
2/Lt. H. W. A. ..	3,750	34	Headache and throb.	16 (solo)	5,000 (as observer) 3,000 (as pilot)	1,000–2,000	Headaches — worse after one hour; has had concussion. By bag method, 'oxygen want' at 4,000.
Capt. P. S. M. ..	3,700	36	Little dizzy; warm in head.	30	8,000	2,000	Goes dizzy and sick in air, with bag method. Broke down at 4,000 ft.
2/Lt. D. W. S. ..	3,500	21	Sort of funny.	2 (solo)	—	—	Dizzy every time he went up.
2/Lt. H. G. G. ..	3,700	40	Head bursting, especially at temples.	P.U.I.	—	—	Bad pressure in head, and discomfort, especially about 2,000.
2/Lt. E. A. P. ..	3,400	45	Rather dizzy.	P.U.I.	—	—	Sick and dizzy in air—2,000–3,000 ft. (probably partly nervous).
2/Lt. S. B. M. ..	2,800	30	Felt little giddy.	Just got wings.	5,000	—	Feels giddy and faint above 5,000. By bag method broke down at 4,500 ft.
2/Lt. J. B.	3,300	21	Little giddy.	8	2,000	—	Giddy above 1,000.

2/Lt. D. K. L.	..	3,000	55	Felt heart.	P.U.I.	8,000	—	Dizzy and headache, 5,000–6,000 ft. By bag method broke down at 5,500 ft.
2/Lt. E. W. C.	..	3,200	26	Uneasy.	25	12,000 (once as observer)	—	Solo, feels faint, over 2,000 'oxygen want' and 'nerves'. By bag method broke down at 8,500 ft.
2/Lt. J. P. W.	..	3,100	45	Head seems to swell.	60	10,000 (once as observer)	—	At 6,000 gets less alert, then not all there. With bag method went very dazed and reached 12,000.
2/Lt. V. P. J.	..	2,700	15	Blood rushing to temples visible.	P.U.I.	2,000	—	Gets headache and blood rushing to temples at 2,000. Was stood off flying, now P.U.P.
2/Lt. G. W. ..	..	3,200	20	Pressure in head and temples.	Just got wings.	8,000	—	Above 5,000–6,000 ft. pressure in head and temples; blurring of vision. With bag method broke down at 5,000 ft.
2/Lt. G. N. ..	..	3,200	35	Queer in head.	P.U.I.	—	—	Giddy during first flight; with bag broke down at 4,500 ft.

In normal pilots it was found that the breath is usually held about 10 to 20 seconds less than before, but always more than 30 seconds. On the other hand, subjects who experienced symptoms, possibly attributable to oxygen-want in the air, usually held their breath less than 20 seconds after such exercise.

With further work the importance of an efficient respiratory capacity in the flying officer was indicated by a number of observations made in the examination of a large number of pilots who were stated to be suffering from stress of service after many hours' flying.

To quote a few such observations, it was noticed that :

(1) Pilots suffering from stress of service flying frequently had a vital capacity which was obviously too small for a healthy adult, e. g. 2,500 c.c., but try as he would the subject could never show what might be deemed a normal capacity.

(2) Although in measuring the vital capacity a good meter was used which opposed but a small resistance, a ' fatigued ' subject frequently had to be encouraged to ' blow hard ' ; in many cases, indeed, the effort to do so rendered him for the time being flushed and out of breath. If not cautioned, a normal subject tends to overshoot the measuring capacity of the meter.

(3) In the ' bag ' method for estimating altitudes (already described, p. 14), the subject is asked to blow up a five-litre bag to distension. Subjects suffering from stress were noticed in many cases to experience difficulty or inability to do this.

(4) In pilots suffering from stress the power to hold the breath was decreased below the normal, to 25–40 seconds. Unlike, however, subjects who suffer from ' oxygen want ' early in their career, these in several cases did not give an abnormal answer such as ' I became giddy ', &c., but gave a normal answer.

(5) With the onset of ' fatigue ' the musculature of abdominal wall shows signs of loss of tone (see Flack and Bowdler, p. 86).

The observations recorded above suggested a loss of expiratory force and possibly some ' fatigue ' of the respiratory mechanism generally. Gradually the following tests were devised to elucidate these points :

(1) The subject was asked to blow a column of mercury contained in a simple U tube to as high a point as possible, the degree of expiratory force being measured in millimetres of mercury.

(2) Measurement was made of the time during which a subject can, after full expiration and full inspiration, sustain by blowing, with the breath held and nose clipped, a column of mercury 40 mm. high.

(3) The vital capacity was measured by getting the subject to fill his lungs and expire as deeply as possible through a good make of meter.

(4) The supplemental air was determined by asking the subject to breathe in quietly and out quietly as in normal respiration, and then to blow his reserve of air through the meter.

(5) The complemental air was determined by a reverse process, namely, after a quiet inspiration to suck in as much air as possible through the meter. Owing, however, to the difficulty of cleansing the tubes, &c., the determination of the complemental air was abandoned as unhygienic, since a number of the subjects were suffering from colds. It was found, however, that, unlike the supplemental air, the complemental air was not affected, except possibly in one case of extreme fatigue.

(6) In a number of cases the behaviour of the pulse during the sustaining of 40 mm. of mercury in expiration was observed, and in some cases recorded by the Mackenzie polygraph.

(7) The period of ability to hold the breath after full expiration and full inspiration was noted in all instances.

Generally speaking, therefore, the routine procedure of the examination was to ascertain the following particulars :

(1) Name and rank.
(2) Age.
(3) Number of hours flying.
(4) Maximum height of flying.
(5) Average height of flight.
(6) Time breath held in seconds after full expiration and full inspiration.
(7) Vital capacity.
(8) Supplemental air.
(9) Expiratory force.
(10) Time during which 40 mm. of mercury sustained by blowing with the breath held.
(11) Other notes of flying career and remarks.

Sufficient time was allowed to elapse between the various tests.

C. Notes on Procedure in Applying Tests.

In the course of the examination there emerged certain points to which particular attention should be paid.

Holding the Breath.—In the 'Holding the Breath ' test the subject was particularly asked to expire audibly as deeply as possible before filling the chest, and the extent of this expiration was noted. While holding the breath the subject was watched and the degree of suffusion of the face, &c., noticed. Inquiry was also made as to the reason for ceasing, and any abnormal answer recorded. The subject must not be told the significance of the test, nor the time he is expected to hold his breath. On no account may he time himself.

As a result of many examinations it has been found that if the subject states that he has done his utmost, is ' all out ', there is little point in asking him to repeat the test. In cases of doubt, however,

a repetition of the test has been made after an interval of several minutes.

Vital Capacity.—In measuring the vital capacity, the subject was asked to fill his chest and blow through the meter. Note was made of the manner in which this was done ; for instance, whether he blew vigorously and overshot the capacity of the meter, or whether he seemed to experience some difficulty in blowing, leading to a suffusion of the face and breathlessness.

As a rule, three determinations were made, in which the subject expired gently and steadily. In practice it was generally found that the second or third reading would be the maximum recorded.

Supplemental Air.—In taking the supplemental air *at least* three estimations were made, the subject being warned to inspire and expire as normally as possible before blowing through the meter.

Expiratory Force.—In regard to the test for expiratory force, the subject was asked to blow the column *steadily* up to the maximum height and not to swing it up by the momentum of the mercury. As the result of experience it has been found sufficient to take two readings with the column turned away and one with the candidate looking at the column. In the experiments here recorded, however, the subject looked at the column in the first attempt. It was borne in mind that in certain cases the value of the test might be negatived by the subject not trying his best. In such cases, therefore, after the first effort, during which he can see the height to which he has blown, the scale of the manometer is turned away, and he is asked to repeat the performance. Generally speaking, the two performances tally, but when it is suspected that the subject is not trying, he is told to blow harder because he can do better. In such cases the result may well exceed the previous effort. After a rest the manometer is then turned with the scale towards the subject, and he is again asked to blow as much as possible. The person who is not trying will probably not exceed the original limit, which he saw registered.

By experiments on himself the writer has found that ' triers ' and ' non-triers ' can be picked out. He has found that, supposing he decides to blow up to 60 mm. Hg when he sees the manometer, he is in a quandary when the manometer is turned away, and cannot then assess 60 mm. with any degree of accuracy.

The blowing power of the cheeks may be ruled out by asking the subject to hold his cheeks with his hand and blow only with his chest and abdominal muscles. Generally speaking, however, the cheeks make little or no difference to the reading.

Sustaining 40 mm. Hg by blowing. ' Fatigue Test.'—In regard to the test of holding up 40 mm. Hg with the breath held, the subject is asked to breathe out as far as possible, and then to fill up the chest before blowing up the mercury. The degree of suffusion of the face and the subsequent breathlessness induced are observed ; also inquiry is made as to the sensations experienced before the breaking point is reached. Usually these are identical with those experienced during the test of holding the breath. It is well to have the nose clipped during the test.

When the pulse rate is taken during the test, the normal rate is previously ascertained by counting it in periods of five seconds before

TABLE II

No.	Name and rank.	Age.	No. of hours flying.	Maximum height.	Average height.	Time breath held in secs.	Vital capacity in c.cs.	Supplemental air in c.cs.	Expiratory force in mm. Hg.	Time in secs. of supporting 40 mm. Hg by blowing.	Remarks.
1	Capt. C. B. G. ..	24 8/12	71	12,000	3,000	67	4,900	1,700	100	67	Instructing practically all time.
2	Capt. D.	26	750	14,000	3,000	46	3,500	1,900	100	51	Instructing 7 months. Before that France 18 months.
3	Capt. H.	32	650	15,500	2,000	105	5,020	1,500	180	48	Egypt 11 months. No illness.
4	Lt. W. H. ..	19	200	15,000	2,000	72	5,050	2,100	90	45	Not been overseas.
5	Lt. D.	29	54	10,000	3,000	56	3,200	—	80	47	Not been overseas.
6	Capt. H.	25	400	13,000	4,000	68	4,400	—	140	52	In France 5 months. Instructing.
7	Lt. H.	21	70	11,000	4,000	64	3,850	—	90	53	Wounded in head in Infantry. Gets dim vision after long flying.
8	Lt.-Col. C. F. K	34 3/12	600	19,000	9,000	47	3,700	1,300	110	43	Still flying, 2 years active service, Mediterranean.
9	Fl. S/Lt. H.A.H.	18 11/12	60	12,200	4,000	68	3,500	1,100	140	48	Recently qualified as Instructor.
10	Fl. Com. S.H.H.	30 8/12	740	15,000	4,000	75	4,000	1,800	120	60	Practically always instructing.
11	Fl. S/Lt. G.R.H.	25 8/12	60	13,000	3,000	53	3,800	1,500	140	45	Instructing only.
12	Fl. S/Lt. G.R.A.	29 8/12	64	11,000	4,000	83	4,300	1,800	110	62	Instructing only.
13	Fl. Lt. L.M.B. ..	23 3/12	700	16,000	3,000	53	3,800	2,000	85	53	Instructing last 13 months.
14	Capt. L. R F ..	26	200	10,000	2,000	66	4,000	—	140	52	—
15	Lt. D. J. A. ..	30 10/12	70 *o* 85 *p*	14,000	12,000 *o* 3,000 *p*	63	4,600	1,800	130	51	Instructing 3 months, from France to train as pilot.
16	Capt. H. L. ..	23	550	14,500	5,000	63	3,700	1,600	100	51	Artillery pilot from France, May 17, instructing since.
17	Lt. A. W. V. ..	28	170 *o* 90 *p*	14,500	5,000	70	3,300	1,200	160	60	—
18	Maj. R. A. A. ..	24 6/12	500	16,000	6,000	51	4,050	1,700	110	54	Little flying lately.
19	Lt. L. M. T. H.	21 2/12	700	18,000	7,000	88	4,100	1,050	80	50	8 mths. instructing. Developed nerves after 6 weeks on S.E. 5 in France.
20	Lt. J. S. T. ..	22 6/12	140	17,500	8,000	63	4,300	1,700	80	45	Recently passed fit for instructing.
21	Capt. D. C. ..	26	320	15,000	7,000	92	4,500	1,700	110	75	—
22	Lt. S. H. W. ..	25	100	22,000	17,500	64	3,800	1,800	80	45	Instructing since October.
				Average ..	..	67	4,062	1,620	112	52	

o = Observer. *p* = Pilot.

TABLE III

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg by blowing.*	*Remarks.*
1	Capt. H.	22 10/12	250	23,000	10,000	125	4,600	1,800	85	56	Home Defence 11 months.
2	Lt. R. N. H. ..	27 6/12	330	22,000	10,000	77	3,900	1,400	80	57	Home Defence 4 months. Before that served in France 6 months. Home for rest.
3	2/Lt. J. A. S. ..	22 3/12	38	6,000	1,000	90	4,300	1,600	185	85	Recently started Home Defence.
4	2/Lt. J. L. W. ..	18 7/12	100	16,000	4,000	73	4,300	1,600	125	62	Home Defence only.
5	Capt. G. H H...	25	700	21,000	8,000	68	3,800	1,400	85	40	Home Defence 5 months. Ferrying 7 months. Before that Scout in France 9 months.
6	2/Lt. J. C. ..	19 4/12	350	19,000	10,000	52	4,200	1,800	180	50	Home Defence 5 months. Before that Scout in France 200 hours.
7	2/Lt. R. M. F...	19 4/12	350	22,000	10,000	63	3,850	1,600	90	38	Home Defence 4½ months. Before that Scout in France 250 hours. Home for rest.
8	Capt. C. J. Q. B.	24 8/12	500	20,000	10,000	85	3,800	1,400	100	50	Home Defence 8 months. Before that Scout 5 months in France (wounded).
9	2/Lt. W. A. P...	20 2/12	250	20,000	15,000	63	3,100	1,000	70	36	Small physique. Home Defence 1 month after 1 month's leave. Before that Scout in France.
10	2/Lt. C C. B. ..	24 1/12									
11	2/Lt. L. F. L. ..	19 6/12	220	17,000	4,000	92	3,800	1,700	140	57	Home Defence 7 months.
			95	17,500	6,000	70	4,200	1,800	125	53	Home Defence since obtaining wings.
12	Lt. R. G. H. A.	21 1/12	120 *o* 125 *p*	16,000	5,000	85	4,800	1,800	90	68	Home Defence 6 months. Before that light duty in France 6 months. Observer (average 12,000 feet).
13	2/Lt. H. A. E. ..	19 2/12	120	19,000	10,000	70	4,300	1,200	145	59	Home Defence 8 months.
14	Capt. D. J. B. ..	25 2/12	650	18,500	10,000	66	4,500	1,800	140	59	Home Defence 5 months. Instructing 1 month. 9½ months France on Martinsydes (av. ht. 14,000) 250 to 399 hours. No illness

15	2/Lt. W H. ..	23	11/12	300	16,500	10,000	66	4,100	1,400	100	53	Home Defence 6 months. Had been up 30 ms. morning of examination.
16	2/Lt. D. G. L. ..	19	3/12	80	12,000	2,000	77	3,100	1,200	120	66	Home Defence 5 months. Had been up 20 ms. on morning of examination. Crashed and temporarily concussed 2 months ago.
17	2/Lt. T. R. B. ..	20	6/12	36	12,000	2,000	75	3,700	1,300	110	63	Home Defence 7 weeks.
18	2/Lt. F. D. H. ..	27	4/12	45	15,000	2,000	71	4,200	1,900	196	57	Had been up 29 ms. on morning of examination.
19	2/Lt. M.H.G.L.	22	7/12	45	15,000	2,000	64	3,700	1,100	110	42	Had been up 25 ms. on morning of examination.
20	2/Lt. L. F. W...	21	3/12	140 *o* 37 *p*	22,000	15,000	65	3,600	1,300	140	47	In high flying squadron as observer in France.
21	Lt. D. V. A. ..	20	6/12	700	21,000	4,000	72	3,800	1,600	130	44	Had been up 1 hour 'stunting' on morning of examination, 280 hours in France. Came home for rest.
22	Capt. J. S. P. ..	22	5/12	150	16,000	2,000	61	3,500	1,300	120	32	'Shortwinded these days.' Not before taking up flying.
23	Lt. W. H. A. ..	25	11/12	170	17,000	10,000	31	3,300	1,300	80	30	Home Defence 7 months. Egypt 2 months. Dysentery Gallipoli. *M.O. wishes him to stand off flying.* Had been ½ hour on observation morning of examination.
24	Lt. G. W. G. ..	22	7/12	150 *o* 420 *p*	22,000	15,000	62	4,100	1,600	105	38	Whenever possible has been to 16,000 twice daily during past 7 months. 'Says he wants leave.'
	Average						72	3,940	1,490	119	50	

o = Observer. *p* = Pilot.

the test is made; then, starting from the moment the mercury reaches 40, counting it every five seconds during the period through which the mercury is sustained.

D. Normal Standards.

The normal standard for the various tests has been set by the examination of a considerable number of selected fit flying officers. In addition, a number of candidates and United States cadets have been examined.

Table II (p. 101) gives the results obtained on fit squadron commanders, flight commanders, and instructors.

Table III (pp. 102 and 103) gives the results obtained on fit pilots engaged in Home Defence (night flying).

It will be seen that the ability to hold the breath averages from 66 to 72 seconds, about the same as that found in the previous examination of successful pilots, e. g. 66 seconds.

The vital capacity is about the same as that found previously, e. g. 3,900 c.c., and the supplemental air is approximately the normal text-book figure, e. g. 1,500 c.c.

The expiratory force averages 112 to 119 mm. Hg, and the average period of holding the breath while blowing up 40 mm. Hg is 52 seconds approximately.

For the sake of comparison, the figures obtained on 24 cadets taken at random from among those passed as fit by the Candidates Board at Arkwright Road are here given in Table IV:

TABLE IV

No.	*Name.*	*Age.*	*Time breath held in seconds.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in seconds of sustaining 40 mm. Hg by blowing.*
1	C. D. G. H. ..	18	84	3,850	1,800	80	45
2	W. N. M. ..	18	70	2,900	1,100	120	45
3	W. E. P. ..	17 11/12	90	4,000	1,800	110	57
4	H. S. H. ..	17 11/12	88	4,200	1,800	80	59
5	E. R. P. ..	18	70	4,100	1,700	110	44
6	R. R. M. ..	18	62	3,700	1,150	110	51
7	J. S. H. ..	17 11/12	62	4,500	1,800	120	52
8	W. H. B. ..	17 11/12	77	3,600	1,600	100	60
9	W. T. P. ..	19 2/12	57	3,800	1,700	120	49
10	D. R. C. ..	19	61	3,400	1,200	120	51
11	H. B. ..	18 1/12	68	4,200	1,800	80	48
12	F. H. ..	21 10/12	52	3,600	1,600	100	44
13	F. T. ..	18 3/12	58	3,700	1,500	90	50
14	2/Lt. P. D. ..	26 2/12	89	4,200	1,800	100	58
15	2/Lt. V. L. W.	21 11/12	77	4,000	1,600	80	51
16	A. F. P. ..	18 2/12	56	3,600	1,600	120	47
17	S. G. S. ..	18 4/12	69	3,700	1,600	90	55
18	G. C. ..	18 2/12	66	4,200	1,700	100	46
19	H. G. G. ..	22 2/	71	3,700	1,600	90	63
20	D. W. ..	23 4/12	70	3,900	1,600	150	60
21	W. S S. W. ..	19 8/12	68	3,800	1,600	120	46
22	H. F. J. ..	18 1/12	60	3,600	1,200	160	52
23	D. E. H. ..	18 1/12	55	3,900	1,700	90	47
	Average		69	3,823	1,593	106	51

It will be seen that in expiratory force and the power of sustaining 40 mm. Hg, they are a little below the previous standard shown on Tables II and III.

It must be remembered, however, that this is a random sample, and the standard of physique of recruits is not now so high as formerly.

Of interest also are the results obtained upon seven United States cadets, from among those now in training in England, who have passed the official tests of the United States Medical Service.

It will be seen that the average is again about the same as the standard found in the fit pilots examined.

From the above normal standards it would seem that the average standard of a flying officer should be :

Time of holding breath ..	66 seconds.
Vital capacity	3,800 c.c. (approximately).
Expiratory force	110 mm. Hg.
Time of sustaining 40 mm. Hg by blowing	52 seconds.

In Table VI (p. 107) are given the results obtained upon a number of delivery and test pilots.

It will be seen that in these the average falls below the normal standard. This may be accounted for by the fact that included in the number of subjects examined are some pilots who suffer from disability at high altitudes, and for this reason are now being employed as delivery pilots. This being the case, it would be expected (as is found) that the average results obtained from these pilots would be below normal. It will be seen, however, that the pilots suffering from marked disability were picked out by these tests.

The value of these tests became evident from the examinations conducted at the first three aerodromes visited.

At the first aerodrome all instructors available were examined, and no questions asked before the examination ; the details of medical history were taken by the Aerodrome Medical Officer, and not by the writer. An examination of the results then showed that all the fit officers had an average for the expiratory force test of 80 mm. or over, and for the sustaining the mercury test of 45 seconds or over. All the officers below this were either unfit for general service or resting after recent high flying in France.

These results are shown in Table VII (p. 108).

At the second aerodrome the medical officer stated that he had two officers unfit for flying. It was agreed, therefore, that they should be examined with the other pilots to see if they could be picked out by these tests.

The results of the examination of the first eight subjects are given in Table VIII (p. 109).

A scrutiny of the results reveals at once that Nos. 4 and 7 were probably the unfit pilots, which actually was the case. The figures also suggest that No. 4 was less unfit than No. 7, which also was true. No. 2 was a pilot of very small physique, recently home from scout flying in France and in need of a rest.

In reality, these two cases (Nos. 4 and 7) illustrate the relative

TABLE V

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of holding 40 mm. Hg by blowing.*	*Remarks.*
1	Cadet W. C. S...	6/12	7 *d*	3,000	2,000	60	3,400	1,350	160	45	Learning with Home Defence squadron. Passed official U.S. examination.
2	Cadet W. F. H. .	22 10/12	4 *d*	2,000	1,000	60	3,600	1,000	120	46	Ditto.
3	Cadet C. K. ..	26 10/12	8 *d*	2,000	1,000	59	3,600	1,050	105	43	Ditto.
4	Cadet G. R. T...	23 6/12	6½ *d*	2,000	1,000	67	3,900	1,600	120	60	Ditto.
5	Cadet C. B. I. ..	24 4/12	6 *d*	5,000	1,000	79	3,900	1,300	100	69	Ditto.
6	Cadet R. B. B...	21 4/12	31 *s*	12,000	4,000	74	4,400	1,700	100	47	Training with Scout squadron. Been up in morning, examined in afternoon.
7	Cadet L. K. C...	23 6/12	4 *d* 4 *s*	3,000	1,000	65	3,900	1,700	110	65	Training with Scout squadron.
				Average ..	..	66·2	3,814·3	1,386	116·4	53·5	

d = *Dual.* *s* = *Solo.*

TABLE VI

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of holding 40 mm. Hg by blowing.*	*Remarks.*
1	Lt. E G. M. ..	28 9/12	450	15,000	6,000	60	2,800	—	100	44	Very small physique, does not like flying high.
2	Lt. T. C.	27	350	18,000	10,000	55	3,800	—	140	43	
3	Lt. F.A.V.W. ..	22 11/12	350	17,000	8,000	17	3,400	—	160	15	Cannot fly above 3,000 now. Killed since. Machine got out of control.
4	Lt. O. M.	25 3/12	200	16,000	4,000	65	3,600	—	70	40	
5	Lt. J. H R. S. ..	22 3/12	450	19,000	6,000	78	3,700	—	90	38	
6	Lt. R. M.	25 9/12	300	20,000	12,000	63	4,500	—	90	43	
7	Lt. H. T.	21 1/12	120	16,000	4,000	53	3,000	900	140	47	
8	Lt. J. E. E. ..	20 9/12	700	8,000	3,000	37	3,800	1,100	85	27	Always disability at height. Fainted at 8,000. Ferry piloting 400 hours.
9	2/Lt. W. P. W. .	20	200	17,000	3,000	67	3,900	1,000	105	51	
10	2/Lt. G. S. H. ..	24 11/12	200 *o* 60 *p*	10,000	5,000*o* 3,000*p*	74	3,700	1,200	105	51	
				Average ..	..	57	3,620	1,050	108	40	

o = Observer. *p* = Pilot.

TABLE VII

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of sustaining 40 mm. Hg by blowing.*	*Remarks.*
1	Capt. H.	25	400	13,000	4,000	68	4,400	—	150	58	Fit.
2	Lt. S.	23	150	15,000	5,000	71	4,100	1,900	100	55	Fit.
3	Lt. H.	21	70	11,000	4,000	64	3,850	—	90	53	Fit.
4	Capt. D.	26	750	14,000	3,000	46	3,600	1,900	100	51	Fit.
5	Capt. H.	32	650	15,500	2,000	105	5,020	1,500	180	48	Fit.
6	2/Lt. D.	29	54	10,000	3,000	56	3,200	—	80	47	Fit.
7	2/Lt. W.	19	200	15,000	2,000	72	5,050	2,100	90	45	Fit.
8	Lt. C.	24	380	10,000	2,000	45	4,500	1,500	130	38	Unfit G.S. France, 6 months. Artillery, 3 months. R.F.C. shell concussion. Sick while flying in France.
9	Lt. M. C.	26	200	10,000	2,000	46	4,000	1,500	70	35	Unfit G.S. Gallipoli, enteric, dysentery, jaundice.
10	Lt. S...	21	500	18,000	5,000	52	3,700	1,000	80	27	'Flying Tired.' Now gets very tired after flying.
11	2/Lt. P.	31	150	18,000	10,000	50	3,700	700	80	27	Resting after high flying in France.
12	Capt. X.	37	800	12,000	2,000	35	4,300	—	120	26	France, East Africa, malaria (C_2H_5OH).
13	Capt. C.	24	150	18,000	3,000	65	4,100	1,200	65	25	Gallipoli, dysentery, enteric, jaundice. Unfit G.S.
14	Lt. B	22	300	21,000	8,000	33	3,300	—	60	24	Been flying (av. 20,000). Recently in France on D.H. 4. Home for rest.

TABLE VIII

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of sustaining 40 mm. Hg by blowing.*	*Remarks.*
1	Capt. A J.Q.B.	24 8/12	500	20,000	10,000	85	3,800	1,400	100	50	Back from France 1st May /17. Scout Home Defence since. 5 months in France (wounds). Before that 2-seaters.
2	Lt. W. A. P. ..	20 2/12	250	20,000	15,000	63	3,100	1,000	70	36	Small physique. From France Nov. /17 for rest. Had 1 month's leave. Been high-flying since 1st July, 1917.
3	Lt. G. C. B. ..	24 11/12	220	17,000	4,000	92	3,800	1,700	140	57	Home Defence scout since June /17. 100 hours; average height 4,000–5,000.
4	Lt. C. S. T. L. ..	21 6/12	125 *o* 200 *s*	22,000	13,000	63	3,550	1,600	125	23	125 hours on flight at 10,000 in France. Left March /17. Leave Nov. 8. Taken off flying by M.O. on day previous; been crashing and making bad landings.
5	2/Lt. L. F. L. ..	19 6/12	95	17,500 *o*	6,000	70	4,200	1,800	125	53	Scout Home Defence since wings.
6	Lt. R. G. H. A.	21 1/12	120 *o* 125	16,000	5,000	85	4,800	1,800	90	68	Scout Home Defence 6 months. Before that light duties in France, Dec. /15—May /16. 12,000 observing.
7	Lt. E. M. G. ..	26	150 *o* 150	16,500	5,000	37	3,600	750	45	23	Taken off flying; been to Dr. Swan previously. Scout Home Defence since July /17. Before that B.E. 12. Observing July /15—Jan. /16. Been flying Jan./16—April /16. Then under Drs. Swan and Head Feb. /17.
8	Lt. H. A. W. ..	19 2/12	120	19,000	10,000	73	4,300	1,200	145	59	Scout since July /17. Home Defence since April /17.

o = Observer. *s* = Solo.

value of the tests, namely, that a flying officer suffering from a degree of stress rendering it undesirable for him to fly, will show this in the first place through the test of sustaining 40 mm. Hg. When the flying stress has become more marked, then it is probable that the ability to hold the breath, the vital capacity, the supplemental air, and the expiratory force will also become diminished. Each of these officers has since been examined, and the results are given elsewhere (Table XIX).

In Table IX (p. 111) are given the results obtained at the third aerodrome, an Acceptance Park.

Scrutiny of the results shows that, judging by the standards of fit subjects, one is able at once to pick out the unfit officer.

Case 6 is particularly interesting. He was examined under the impression that he was a particularly fit and experienced officer. He had been posted to the aerodrome the day before, and reports of his past good work had preceded him. It will be seen that the result of the examination was most unsatisfactory, and on inquiry it was elicited that he had been stopped flying at his last aerodrome one month before on account of stress of service. He expressed himself as still thoroughly ' fed up ' with flying ; and undoubtedly after 900 hours in the air, with 23 days' leave in three years, he was a good example of flying stress.

With a view to determining the value of the tests as a measure of fatigue, a number of pilots who had returned for rest from active service overseas were examined (Table X, pp. 112 and 113).

All these pilots were deemed sufficiently fit to be posted for flying at home ; in fact, all were examined at aerodromes where they were flying. It is seen that on the average, especially as regards expiratory force and ' sustaining mercury ', they fall considerably below the normal standard.

From the point of view of fatigue also a number of pilots training on scout machines were examined (Table XI, p. 114).

It will be noticed that the expiratory force and ' sustaining mercury ' is below the average. Scrutiny of the table, however, reveals the fact that this is due to a certain extent to the performances of some of the subjects rather than to a low general standard.

In regard to the subjects who did badly, the writer considered that *from the point of view of wear and tear*, they were not likely to do well on scout machines, an opinion which was in every case shared by the officer commanding or the flight commander. *It would seem, therefore, that a routine application of these tests to pilots in training for high flying would indicate the powers of endurance of the subject for the fatigue of high flying.*

This is further brought out by the fact that it has been found that a flight, even in some cases to low altitudes, tells considerably more on some subjects than on others. Thus a very fine scout pilot examined after an hour of most amazing flying satisfied the tests (see No. 21, Table III). On the other hand, it is common to find that a pilot just down after ' stunts ' or even from a straight flight is unable to hold his breath 40 seconds or sustain the mercury at 40 mm. Hg more than 25 seconds. The vital capacity is also greatly lessened, being frequently under 3,000 c.c. After resting for half an hour or so he will return to

TABLE IX

No.	*Name and rank*	*Age.*	*No of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of sustaining 40 mm. Hg by blowing.*	*Remarks.*
1	Lt. H. T.	21 1/12	120	16,000	4,000	53	3,000	900	140	47	Wooden leg. Delivery 3 weeks. Before that instructing. Home service.
2	Lt. J. E. E. ..	20 9/12	700	8,000	3,000	37	800	1,100	85	27	Fainted 8,000 after about 60 hours. Always disability at height. No A.B. examination. Ferry piloting since March (400 hours).
3	2/Lt. W.P.T.W.	20	200	17,000	3,000	67	3,900	1,000	105	51	Ferry pilot since July. Average 3,000. Instructing 1 month. France Scout 4 weeks (shot down), 3 weeks on pool.
4	Lt. R. S. H. ..	23 11/12	200	13,500	2–3,000	91	4,300	1,700	80	42	Off regular flying—stood off by O.C.'s instructions. Limited flying only. Overseas 4 months. Art. obs. av. 7,000 (100). Crashed, now some dizziness.
5	Lt. G. H.	24 10/12	200 *o* 60	10,000	5,000 *o* 3,000	74	3,700	1,200	105	57	Art. observer in France. Delivery pilot. Not had any disability.
6	Lt. W. H ..	29 7/12	900	16,000	7,000	23	2,600	700	50	20	Delivery for 9 months 1,000 feet average. Testing aeroplanes 18 months 20,000—10,000. *Stopped flying by M.O.'s orders at last aerodrome. Now 'fed up'.* 23 days leave in 3 years. Gets winded if runs 100 yards.
7	Lt. W. F. W. ..	20 6/12	350	20,000	10,000	40	3,250	1,200	110	37	Limited flying. Back from France (Nov. 1). Scout 80 hours. 20,000 at first but not towards end. Sent home for rest Practically fainted at 18,000. In 1916 was flying Moranes at average 7,000 quite O.K

o = Observer

TABLE X

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height of all flying.*	*Time bre th held in secs.*	*Vital capacity in c.cs.*	*Supple-mental air in c.cs.*	*Expira-tory force in mm. Hg.*	*Time in secs of holding 40 mm. Hg by blowing.*	*Remarks.*
1	Lt. B. McE. ..	26 2/12	230	20,000	8-10,000	47	3,800	1,300	60	45	France 6 months on Spads. Home 3 months. Now instructing.
2	Capt. W. E. Y.	26	300	20,000	10,000	65	5,400	2,100	100	35	France Scout 2 months. Average height 10,000 ft. Back 5 months. Flying all the time since.
3	Capt. R. L. K.	25 8/12	500	23,000	5,000	49	3,400	1,000	70	34	France Scout 6 months. Average 14,000. Before that bombing (average 11,000). Now instructing.
4	Lt. P.	31	150	18,000	10,000	50	3,700	800	80	27	France 9 months, 3 months on Scouts (average 16,000). Now instructing.
5	Lt. B.	22	300	21,000	8,000	33	3,600	—	60	24	France 4 months D.H. 4 (average 19–20,000). Now instructing. Still some nervous tremor.
6	Lt. T. C.	27	350	18,000	10,000	55	3,800	—	140	43	France Scout (D.H. 2 and D.H. 5). Average 14,000. Now delivery pilot.
7	Lt. J. H. R. S.	22 3/12	450	19,000	6,000	78	3,700	—	90	38	France Scout 6 months. (D.H. 2 and Nieuport.) Average 14,000. Now delivery pilot.
8	Lt. R. M.	25 9/12	300	20,000	12,000	63	4,500	—	90	43	France 6 months, D.H. 4 and Sopwith Scouts. Back 6 months. Now delivery pilot.
9	Capt. C. D. D. ..	30 6/12	750	15,000	4,000	57	3,500	1,500	120	45	France 6 months Scouts. Average 12–14,000. Back 10 months. Now instructing.
10	Lt. R. M. F. ..	19 11/12	350	22,000	10,000	63	3,850	1,600	90	38	France 250 hours, Sopwith Scout. Last 4½ months Home Defence.
11	Capt. G. H. H.	25	700	21,000	8,000	68	3,800	1,400	85	30–40	France 9 months, 340 hours on Scout. Since then ferrying 7 months. Home Defence 5 months. No leave on return from France for rest.

12	Capt. J. D. B. ..	25 6/12	650	18,500	10,000	66	4,500	1,800	140	59	France 9½ months Martinsydes. Average 14,000. Since then instructing one month. Home Defence 5 months.
13	2/Lt. W. A. P.. .	20 2/12	250	20,000	15,000	63	3,100	1,000	70	6	Five months Scout France. Back 2 months, 1 month's leave. Now Home Defence. Height 5 ft. 4 in., Weight, 8 stone.
14	2/Lt. J. C. ..	19 4/12	350	19,000	10,000	52	4,200	1,800	180	50	Home Defence 5 months. Before that Nieuport in France 300 hours. Then sent home for rest.
15	Lt. R. N. H. ..	27 6/12	330	22,000	10,000	77	3,900	1,400	80	57	Home Defence 5 months. Before that Scout France 6 months. Average height 16,000. Then home for rest.
16	Capt. H. C. T. ..	25	300	18,500	5,000	35	3,900	1,700	60	29	In France 9 months on Scout. Back 9 months. Now instructing. Had been up in morning, examined in afternoon.
17	Fl. Lt. L.B.F.W.	19 7/12	275	18,500	12,000	58	3,600	1,100	100	43	Off Scouts for 8 months. Now instructing.
				Average ..	..	57	3,897	1,423	95	40	

TABLE XI

No.	*Name and rank.*	*Age.*	*No. of hours flying*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg by blowing.*	*Remarks.*
1	2/Lt. J. D. H. ..	18 10/12	67	13,000	5,000	66	3,900	1,500	100	55	Training for scout.
2	2/Lt. A. P. H. ..	18 10/12	60	10,000	5,000	55*	3,500	1,250	60	40	Training for scout. *Swimming in head.
3	2/Lt. J. M. B. ..	26 5/12	20	6,000	3,000	95	3,700	1,300	109	71	Training for scout.
4	2/Lt. A. B. G. ..	33 6/12	80	12,000	2,000	71	3,450	1,200	140	47	Training for scout.
5	2/Lt. F. A. ..	21 3/12	15½ *s*	6,000	2,000	45	3,000	950	70	40	Training for scout.
6	2/Lt. B. I. ..	24 2/12	12	5,000	2,000	68	4,200	1,800	110	58	Training for scout.
7	2/Lt. R. J. L. ..	31 10/12	70	15,000	5,000	72	4,850	2,000	100	54	Just finished training for scout.
8	2/Lt. R. A. B. ..	19	95	16,000	5,000	46	3,750	1,600	60	47	Training for scout. Had been up 2 hours in the morning; examined afternoon.
9	2/Lt. L. H. C. ..	18 10/12	40	12,000	3,000	45	3,700	1,200	100	42	Training for scout.
10	Lt. M. L. H. ..	25 3/12	35 180 *o*	16,000	7,000	57	3,900	1,200	120	50	Training for scout. Up 1 hour 15 minutes in morning; examined afternoon.
11	2/Lt. P. J. N. ..	20 2/12	35 180 *o*	11,000	7,000	53	3,400	1,300	80	45	Just qualified for scout.
12	Lt. T. T. S. ..	26	130	13,000	8,000	66	4,500	1,700	130	48	
13	2/Lt. S. C. W. ..	23 6/12	70	16,000	6,000	61	2,850	1,200	85	36	Small physique.
14	2/Lt. K. J. B. L.	27 4/12	60	14,000	5,000	76	4,450	1,700	80	50	
15	Lt. F. L.	25	116 *o* 16 *p*	22,000 *o*	16,000	55	4,150	1,600	119	50	
			Average ..	..	..	62	3,820	1,433	96	49	

s = Solo. *o* = Observer. *p* = Pilot.

about normal standard. Further experiment is required, however, to see what amount of flying is necessary before lasting deterioration takes place and flying stress gradually creeps in. It will undoubtedly be found to vary greatly, as seen above, in individual cases. Some pilots will manage two 'shows' a day with ease, others will not.

The after effect of a straightforward five-hour flight on a delivery pilot is given below; he was a big man weighing over 13 stone, and was not examined until 24 hours after the flight.

Lt. D. W. H. . . Age $29\frac{8}{12}$.
Flying 300 hours.
Maximum height 16,000, average 4,000.
Breath held 80 seconds.
Vital capacity 3,500 c.c.
Supplemental air 1,100 c.c.
Expiratory force 40 mm. Hg.
Sustaining 40 mm. Hg 21 seconds.

In contrast to this case is that of Capt. B. E. B., who served $10\frac{1}{2}$ months strenuous flying on high-flying machines overseas. After thorough examination at Etaples by the late Lieut.-Col. Corbett, who found him practically unaffected by altitudes (Dreyer's method), he was examined at Hampstead with the following result:

Capt. B. E. B., D.S.O., M.C. . Age $21\frac{4}{12}$.
Flying 1,000 hours.
Maximum height 22,000 feet.
Average height 15,000 feet.
Breath held 110 seconds.
Vital capacity 4,250 c.c.
Supplemental air 1,700 c.c.
Expiratory force 180 mm. Hg.
Sustaining 40 mm. Hg 57 seconds.

During the last test the pulse rate gradually increased from 66 to 84 per minute. Such a subject is undoubtedly a super-high altitude flyer. This is particularly brought out by a comparison of the above results with those on pilots who have broken down and have been taken off flying through stress of service, a stress more marked in some cases than others (Table XII, pp. 116–18).

These results show that in such subjects there is a marked falling off in the ability to perform the tests. It will be noticed that the subjects are for the most part high altitude flyers. The two officers (Nos. 10, 22) who almost attain the normal standard in sustaining mercury at 40 mm. with the breath held, had been off flying for some months past.

The value of the tests may be set forth by collecting the *average* results into a table (Table XII).

From the above figures it would appear that for the expiratory force test 80 mm. Hg should be taken as a minimum. For the test of sustaining 40 mm. Hg with the breath held, 40 seconds should be regarded as the minimum time. If below this it would appear advisable to limit flying both as to hours and height, and if much below this figure, to take the subject off flying for a time altogether.

TABLE XII

No.	*Name and rank.*	*Age.*	*No of hours flying.*	*Maximum height.*	*Average height of all flying.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of holding 40 mm. Hg by blowing.*	*Remarks.*
1	Flt. S/Lt. R. F. .	22 6/12	600	22,000	10,000	39	4,000	—	50	15	200 hours France at average 18–20,000. Gradual onset of disability to fly high. Cardio-vascular system good, nerves good.
2	2/Lt. J. K. C. ..	20 9/12	200	21,000	10,000	61	4,000	—	50	19	High flying on D.H. 4. Feels effects after 3 weeks.
3	Lt. J. H. C. ..	29 1/12	400	13,000	10,000	52	3,200	1,400	80	25	Lately delivery pilot. Before that D.H. 2 in France. 'Fed up with flying.'
4	2/Lt. A. H. W.	24 10/12	75	15,000	10,000	63	4,000	1,700	100	37	Scouts in France 4 months. Back 1 week. Fainted at 6,000 after stunting for 1 hour.
5	2/Lt. W. D. ..	26 6/12	100	21,000	12,000	55	3,700	1,000	60	29	Five months Scout. Fainted after coming from 15,000 to 5,000 quickly. No flying for 3 months.
6	2/Lt. G. H. S. ..	24 3/12	170	15,000	12,000	45	3,450	1,000	80	21	On Scout in France 3 months. Back 1 week. Cardio-vascular debility and splashing stomach.
7	Flt. S/Lt. E.F.B.	23 8/12	100	18,000	14,000	40	2,650	450	40	5	60 hours Scout and high flying bombers. Slight physique. Headache and gradual breakdown. Influenza 1 month ago. Off flying 6 months.
8	Capt. C. N. J. ..	21 3/12	200	19,000	12,000	50	3,450	1,600	42	3	France 100 hours Scouts, then wounded.
9	Lt. E. B. G. ..	21 4/12	700	20,500	12,000	43	3,450	600	50	25	450 hours on Scouts in France, then shot down 5 months ago.
10	Capt. R. M. F.	22 8/12	400	18,500	10,000	68	4,000	1,700	120	45	Scouts in France, came home with 'nerves'. No flying for 4 months past.

11	Capt. G. N. M.	23	8/12	460	16,000	12,000	64	3,500	1,100	50	27	Scouts France 100 hours D.H. 5. Before that instructing 5 months. Before that Scout France 8 months. (D.H. 2.)
12	Lt. C. S. L. ..	21	6/12	200 125 *o*	22,000	13,000	63	3,550	1,600	125	23	Lately Home Defence 2 months. Taken off flying by M.O. Before that 5 months Scout France.
13	Lt. E. M. G. ..		26	150 150 *o*	16,500	5,000	37	3,600	750	45	23	Home Defence 6 months. Before that in hospital for nerves and headaches after flying Scouts in France.
14	Lt. W. L.	29	7/12	900	16,000	7,000	23	2,600	700	50	20	Examined after one month's leave. Lately instructing 350 hours in nine months. Before that testing 18 months. Average height 10,000 ft. Now 'fed up with flying' and stood off by M.O.
15	2/Lt. W. F. W.	20	6/12	350	20,000	10,000	40	3,250	1,200	110	37	Back from France for rest 2 months after 80 hours Scouts (average 18,000 ft.). Flying Moranes in 1916. Now starting limited flying.
16	Lt. W. A. W. ..	25	6/12	80	13,000	8,000	54	3,600	1,300	80	27	Back from France 5 days. Scout 40 hours. Now suffers giddiness during stunts, particularly in dives.
17	Capt. S. J. S. ..	29	6/12	300	20,000	14,000	59	3,600	1,300	140	31	Back from France 5 days. Scouts on active service 250 hours. Could not possibly do more in holding Mercury test.
18	Lt. J. J. P. ..	25	11/12	140	13,000	10,000	60	4,100	—	70	35	Shot down 9 months ago while high flying (photos). Now neurasthenia.
19	Fl/Lt. E. J. C...	21	1/12	300	11,000	3,000	44	3,200	600	90	37	Five hours patrol for average 5 days weekly for 6 months past. Before that Scouts one month and seaplane 12 months. Stood off for rest.
20	Lt. T. F. A. ..	22	3/12	1,575	10,000	2,000	60	3,800	1,800	60	33	3 years 6 months service instructing and testing. No leave except sick leave 3 months for damaged spine. Restless.

o = Observer.

TABLE XII (*continued*)

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height of all flying.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of holding 40 mm. Hg by blowing.*	*Remarks*
21	2/Lt. W.A.McC.	19 6/12	110	15,000	4,000	25	3,900	1,600	45	16	Possibly oxygen want. Delivery Pilot 3 months, now stood off. Before that D. H. 4s. France one month, which knocked him up.
22	Capt. F. H. ..	29 6/12	600	14,500	10,000	58	3,100	800	100	45	Off flying 8 months. Flew 4 months with malaria and got absolutely knocked up. Very nervous. Has just started flying a little. Now feels keen.
23	Lt. J. H. C. ..	29 3/12	400	13,000	10,000	52	3,200	1,400	80	25	Flying D.H. 2 in France. Then Test and Delivery Pilot. 'Fed up with flying.'
24	Lt. G. S. W. ..	26 8/12	65	15,000	12,000	42	3,800	—	80	22	Could not stand high flying, two crashes following high flights.
25	2/Lt. C. N. H. ..	21 6/12	50	10,000	5,000	38	2,300*	500†	80	20	Training for Scout. Fainted 10,000 ft. Eight crashes due to bad landings. Admitted A.B. Held breath on admission 43 seconds. Oxygen used 312 c.cs. per kilo per hour.
26	Capt. W. W. H.	28 1/12	500	13,000	5,000	38	3,500	850	50	18	Instructing 6 months. Before that France 250 hours. Average 8,000. Fainted in air. Felt very bad.
27	Lt. C. F. E. ..	33 6/12	200	14,000	6,000	37	3,400	—	75	20	Artillery observation 120 hours. Delivery Pilot since Nov. Now tired after long trips.
				Average ..	..	49	3,480	1,134	74	25	

* Maximum of 4. † Average of 5.

TABLE XIII

Subjects.	*Number examined.*	*Breath held in seconds.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Sustaining 40 mm. Hg.*	*Remarks.*
Fit instructors	22	*67	4,062	1,620	112	52	* Average.
Ditto ..		†46	3,300	1,000	80	43	† Minimum.
Fit Home Defence pilots	24	72	3,940	1,496	119	50	One or two suffering from stress included in the table.
Fit British candidates	23	69	3,823	1,590	106	52	
U.S. candidates	7	66	3,814	1,386	116·4	53·5	
Delivery and test pilots	10	57	3,620	1,050	108	40	
Pilots returned for rest	17	57	3,897	1,423	95	40	
Pilots training for scouts	15	62	3,820	1,433	96	49	
Pilots taken off flying through stress	27	49	3,480	1,134	74	25	

In addition to these subjects, a large number of hospital cases have been examined. The results of many of these are given in Tables XIV and XV (pp. 120–21 and 122–25).

In addition to the poorness of the performance with the 40 mm. Hg 'fatigue' test, the smallness of the vital capacity (e.g. Case No. 13, Table IV) and of the supplemental air is very noticeable in many cases. Taking 80 mm. Hg as the minimum for expiratory force and 40 seconds as the minimum for sustaining 40 mm. Hg with the breath held, it would seem that after injury following crashes, particularly after injury to the head, the subject fails at the tests. This would indicate that the *tests will be of value in assessing the degree of shock sustained by a pilot in so-called 'minor' crashes.* It is of interest that 'psychopaths' (determined by the neurologist) pass the tests quite well, whereas subjects suffering from nervous symptoms due to flying stress fail. With a history of flying after dysentery or malaria, the subjects frequently fail at the tests (cf. also Table XV), confirming the opinion already formed as to the injurious effects of these diseases. It is also of interest that a case of indigestion (No. 11) and a case of blindness in one eye (No. 32), examined without the history being taken, pass the test. In neither subject was there reason to suppose that there was any stress of service. It should be noticed, however, that, generally speaking, almost *all the hospital cases examined (except psychopaths) fail to pass the tests.*

In Table XV there is no attempt to differentiate between officers who came to the hospital suffering from stress of service, and other cases, surgical or medical.

In this table, however, is also included a column showing the pulse response during the time that the mercury is being sustained at 40 mm. with the breath held, otherwise the same remarks apply to this as to Table XIV.

From Table XV it will be seen that the counting of the pulse during the sustaining the 40 mm. Hg test (with the breath held)

TABLE XIV

No.	Name and rank.	Age.	No. of hours flying.	Maximum height.	Average height.	Time breath held in secs.	Vital capacity in c.cs.	Supplemental air in c.cs.	Expiratory force in mm. Hg.	Time in secs. of supporting 40 mm. Hg by blowing.	Remarks.
1	2/Lt. J. R. F. ..	27	80	12,000	2,500	62	3,600	1,300	90	45	Primary psychopath.
2	Lt. J. C.	24 8/12	150 *o* 4 *d* 10 *s*	10,000	6,000	93	3,750	4,200	70	53	Psychopath. Possibly some fatigue.
3	2/Lt. C. F. S. ..	19 3/12	24 *s*	8,000	3,000	38	3,400	800	70	19	Neurasthenia following crash (concussion).
4	P. F. O. F. W. ..	18 9/12	9 *s*	5,000	1,500	52	2,500	1,100	70	37	Resting after crash—bashed head against fusilage.
5	2/Lt. W. R. W.	19 2/12	1 *d*	—	—	92	4,100	1,800	80	50	Asthma.
6	Lt. W. D. ..	26 6/12	100	21,000	12,000	—	3,700	1,000	60	29	Cardio-vascular debility. Fainted after coming.
7	2/Lt. G. L. T. ..	29 1/12	45	8,000	5,000	53*	4,100	2,000	76	40*	Anaemia (very marked). *Giddy.
8	Lt. E. W. C. ..	29 6/12	200	17,500	4,000	50	3,400	1,100	140	43	Back from France 5 days. Night flying. Bronchitis.
9	2/Lt. R. I. W. ..	20 7/12	20min.	—	—	38	2,700	1,100	60	30	Gastric trouble.
10	2/Lt. R. H. T.	19 7/12	20 *d* 2 *s*	3,500	1,000	72	3,450	1,400	50	51	Crashed—hurt foot, always making bad landings.
11	2/Lt. J. S. ..	24 2/12	11 *d* 30 *s*	10,000	5,000	75	3,800	1,000	180	64	Indigestion.
12	2/Lt. R. H. D...	18 5/12	100	14,000	3,000	45	3,400	850	110	40	Tonsillitis. Had a cold at time of examination.
13	Capt. S. E. G. ..	26 2/12	300	12,000	7,500	30	1,500 (3 attempts)	—	45	12	Crashed Feb. /17—concussed. Now dizzy at 5,000 or over. Fainted at 8,000. Test gave him headache. Inspiratory force 60 mm. Hg. Sustaining 40 mm. Hg by inspiring 12 seconds.
14	Capt. A. S. C. ..	24 9/12	150	8,000	2,500	68	4,500	—	60	28	Malaria.
15	2/Lt. G. W. ..	32 4/12	8	1,600	1,000	48	3,100	1,000	100	18	Neurasthenia following crash. Stomach trouble, sickness and diarrhoea after landing.
16	2/Lt. E. T. D. ..	32 5/12	3 *d*	4,000	1,500	42*	3,500	800	38	3	*Giddy.
17	2/Lt. R. W. T...	21 5/12	23	9,500	3,500	45–72	3,500	1,000	80	40	Enteric and sunstroke.

o = Observer. *d* = Dual. *s* = Solo.

18	2/Lt. T. T. ..	28	11/12	300	14,500	9,000	42	3,600	900	80	35	Dysentery. Instructing last 7 months. Before that France 8 months. Average 9,000 ft.
19	Flt.-Lt. F.D.H.B.	24	10/12	150	13,000	10,000	59	4,400	1,600	110	39	No flying 18 months. Trench fever and malaria.
20	2/Lt. E. N. C. ..	19	10/12	5½*d* 1 *s*	2,000	—	30	3,600	1,100	130	22	Cardio-vascular debility. No A.B. examination. Has had malaria.
21	E. T. M.		19	60	5,000	2,000	35	3,200	1,150	50	20	Oxygen want. Throbbing in head. No A.B. examination. Bad landings.
22	2/Lt. P. R. ..	28	9/12	100	15,000	3,500	65	3,500	1,300	105	45	Premature contractions following influenza. Night bombing 25 hours.
23	Flt.-Lt. G. A. M.	21	6/12	175	15,000	10,000	94	4,100	1,300	140	75	Shrapnel wound in head May/17. No flying since.
24	Capt. R. G. M.. .	22	11/12	1,500	23,000	10,000	40	4,100	1,750	60	28	Nerves gone. Has fainted in the air. Limited flying since Dec. /17 due to synovitis of knee. Before that Scout in France (250 hours).
25	2/Lt. D. D. P. ..	20	4/12	75	12,000	3,000	30	3,200	1,100	60	20*	Dilation of heart (influenza), 'nerves', dyspepsia. *Temples turn red.
26	2/Lt. W. B. ..	18	10/12	13 *s*	10,000	2,000	44	3,400	900	60	18	'Nerves' (crash—not unconscious—sent to hospital 4 days).
27	Lt. C. B. L. ..	27	6/12	8 *d*	—	—	44	3,000	1,000	80	35	In hospital since crash (August), unconscious fortnight. Many fractures.
28	Lt. W. B. H. ..	25	9/12	106	9,000	1,000	44	2,600	550	60	25	Instructing M.F. Tonsils, indigestion.
29	2/Lt. L. L. ..	19	3/12	15 *o*	7,000	6,000	29	3,200	200	45	22–19	'Nerves', crash—thrown on head, dozed, then went dizzy. Still comes on dizzy.
30	Capt. A. S. L. ..	23	6/12	400	23,000	15,000	55	4,000	1,300	140	45	Scouts nearly all time. From France Jan. 8, 8 months (300 hours, average 16,000). One leave 14 days beginning November.
31	2/Lt. F. C. ..	21	8/12	200	22,000	16,000	45	3,500	1,300	80	42	From France Jan. 19, 4 months flying Scouts (S.E. 5), 50–60 hours. Run down—home on leave.
32	Capt. G. C. M. ..	34	7/12	250	18,000	12,000	66 (N)	3,800	1,400	130	47	Defective eyesight. Left eye useless. Makes good landings. From France 2 days. Since July flying Scouts in France 150 hours, average height 12,000, before that 'ground-strafing' 2,000 ft.

o = Observer. *d* = Dual. *s* = Solo.

TABLE XV

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg by blowing.*	*Pulse response.*	*Remarks.*
1	Maj. S. G. S. ..	24 7/12	600	15,000	10,000	77	4,500	1,800	110	46	84–132 and maintained	Psychopath.
2	2/Lt. C. W. M...	25 11/12	140	13,000	10,000	46	3,750	1,300	140	41	60–96 and maintained	Last 2 months Italy, 4 months active service, 70 hours, average height 10,000.
3	Lt. P. J. W. R.	24 11/12	40	11,000	10,000	69 (N)	3,500	800	60	22	72–108–60	From France. Enteric 18 months ago. Crashed first time up, 6 weeks in France.
4	Lt. A. N. A. ..	26 11/12	200	19,000	8,000	85 (N)	4,100	1,250	160	65	90–100 and maintained	From Italy and France R.E. 8. Average 8,000, 80 hours. Instructing England. Crash, injury to stomach and head. Nerves gone.
5	2/Lt. W. R. B...	19 5/12	10 *d* 5 *s*	10,000	2,000	41	3,200	1,200	70	24	84–108 and maintained	Blow on head from propeller. Headaches.
6	Lt. H. W. T. ..	21 1/12	65	17,000	10,000	62	3,500	1,000	80	25	78–108–78	From France, D.H. 4, 3 hours. Crash, blow on eye. Headaches since.
7	2/Lt. I. V. H. ..	30 6/12	200* 60†	18,000	7,000	55	3,900	1,800	120	41	72–84 and maintained	Headaches, neurasthenia. Limited flying, not above 3,000 ft.
8	2/Lt. H. O. ..	28 1/12	130 *o*	12,000	2,000	37–49	3,400	1,000	85	19	72–120–96	Artillery shoots. From France. Crash August 12th. Unconscious.

9	2/Lt. D. S. R. ..	20	9/12	100	17,000	14,000	35	3,750	1,150	115	25	72–96–72	From France. Crashed. Unconscious Oct. 2/17. Hospital since. Scouts 30 hours.
10	Lt. B. L. B. ..	23	6/12	100 *o*	10,100	5,000	43	3,350	800	80	35	78–108–96	From France (24). History of crashes.
11	2/Lt. R. C. ..	19	6/12	450	18,000	8,000	46 (N)	4,200	850	64	43	60–72 headaches	Italy R.E. 8. Average 8,000. Photography. Home on leave. Did not feel fit to go back.
12	Lt. B. T.	22	6/12	160	19,500	14,500	72 (N)	4,000	850	120	35	66–96 and varying	From France. In Hospital 1 week. S.E. 5—4 months overseas, 60 hrs. England 100 hrs. Average 4,000. Shell burst at 17,000 over Cambrai. Managed to get back at 2,000.
13	2/Lt. A. C. ..	24	8/12	160	18,000	12,000	50	3,400	800	100	33	66–108–72	From France. Three crashes. Bad landings, S.E. 5, 100 hours. Average 12,000. Training in England.
14	Lt. A. G. G. ..	24	9/12	160	18,500	14,000	52	3,500	1,000	120	34	72–96–72	From France. 'Colossal wind up.' D.H. 4, no oxygen. Average height 16,000. Since July 120 hours. Cannot manœuvre machine as he used, takes him all his time to get into aerodrome.
15	2/Lt. W. O'T. ..	25	3/12	150 *o* 10 *p*	22,000	18,000	40	3,500	—	60	45	72–120 and maintained	Bristol fighter (48 hours) overseas. In hospital for crash, head cut, eye damaged. During training at Hounslow fainted and crashed from 300 ft.

d = Dual. *s* = Solo. * = R.N.A.S. † = R.F.C. *o* = Observer. *p* = Pilot.

TABLE XV (*continued*)

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg by blowing.*	*Pulse response.*	*Remarks.*
16	Lt. N. V. B. ..	21 7/12	250 *o* 190 *p*	20,000	14,000	99 (N)	4,200	1,700	90	48	72–96	From France 10 days, S.E. 5—6 months. (140 hours.) 17,000 average.
17	2/Lt. C. W. S. ..	19 4/12	55 *p*	14,000	5,000	43 (N)	3,700	1,000	50	27	72–96 and maintained	From Italy. 20 hours. Average 4,000. Home for further instruction. 'Nerves' 7 hours ago.
18	2/Lt. H. M. M.	20 1/12	—	—	—	66	4,200	1,800	120	35	84–132 and varying	No flying. Reading. Admitted A.B. Fractured pelvis 9 weeks ago.
19	Lt. C. W. P. ..	29 2/12	50	8,000	4,000	22 (N)	3,750	00	70	23	108–132–96 varying	In hospital, fractured nose. 'Nerves.'
20	Lt. C. R. G. ..	28 9/12	90	15,000	7,000	48 (N)	3,300	1,600	90	47	72–108	Fractured skull, 25 Sept. /17. Scar on face by gun mounting. Lost consciousness 10,500. Next thing staggering about ground. Was going on sick leave.
21	Lt S. C. B. ..	35 6/12	50	10,500	5,000	71 (N)	—	—	100	45	90–132–96 varying	Flying F.E. night. Crashed Feb. 15. Shaken up. Hospital R.E. 8 (8 hours).
22	Lt. F. A. P. ..	26 6/12	80	10,000	5,000	41	—	—	80	25	72–96–72	France S.E. 5 (10 hours). Crashed twice in 3 days. D.H. 5 (10 hours).
23	2/Lt. A. B. ..	21 1/12	5 *d* 5 *s*	6,000	2,000	37	—	—	100	27	72–120–96 varying	Crashed (stammer since). Flying continued. Bashed head with crash helmet.

24	Lt. W. A. R. D.	27 4/12	40 *o*	22,100	2,000	55	—	—	100	35	84–144–132	From France. 8 weeks Bristol fighter overseas. 15–20,000 average. Lost confidence suddenly.
25	Maj. G. A. C. ..	36	800	10,000	3,500	25	2,500	1,200	80 (giddy.)	20 (headache.)	72–784	Dysentery, malaria, flying in all countries, but not feeling fit since illnesses.
26	Capt. M.G.F.R.	25 7/12	650	17,500	5,000	82	4,200	1,600	100	38	84–108 and maintained	From France 4½ months. Night flying (average 5,000) 16 months.
27	2/Lt. J. C. M. ..	19	65	12,500	5,000	61 (N)	4,400	1,800	120	53	84–108–60	Training for scout.
28	2/Lt. E. S. P. ..	21	140	19,500	16,000	30	4,000	1,100	60	15	60–96 and maintained	From France. 5 months. Average 16,500–90 hours. Egypt 3 months. 30 hours average height 8,000.
29	Lt. H. T.	30	250	18,000	10,000	90	3,800	1,020	65	41	72–132–108 varying	Fractured base 8 weeks ago.
30	Fl. S/Lt. F. E. D.	24 7/12	100	16,000	2–3,000	72	3,950	1,800	80	59	78–96 maintained	Perforation ear and fractured base 2 months ago.

o = Observer. *p* = Pilot. *d* = Dual. *s* = Solo.

affords valuable indication of the degree of stability of the cardiomotor centre. It had previously been found that counting the pulse during holding the breath does not give sufficiently striking differences on which to generalize. The late Lieut.-Col. Corbett, R.A.M.C., however, informed the writer that, during the Dreyer altitude test (see Report No. 4) he had noticed a difference in the response of the pulse between normal and abnormal individuals, and observation of the pulse behaviour as a routine procedure while holding 40 mm. Hg was in the main an outcome of this piece of information. From comparison with Col. Corbett's notes it would seem that the results are fairly comparable during the two tests.

The exact significance of the pulse response has yet to be worked out in detail in regard to the various types of hospital cases. Generally speaking, it may be stated that, starting at the 5th second, there is in the normal individual a steady low rise in the rate of the pulse (cf. Capt. B. E. B.), or a fairly marked rise, which is sustained most of the time. For example, the pulse rate may rise gradually from 72 per min. to 96 or 108, according to the time the breath is held, or the pulse may rise almost at once from 72 per min. to 98 or 108 and be maintained there. A large rise in rate, for example, from 72 to 132 or 144, is, generally speaking, unsatisfactory. In the case of Fl.-Lt. B. (No. 7, Table XI), who could only sustain 40 mm. Hg for 5 seconds, it was found that when 20 mm. Hg was held for 10 seconds the pulse rate rose from 96 to 168 per min., becoming almost impalpable.

In cases of flying stress with cardio-vascular symptoms, a characteristic response seems to be for the pulse to jump quickly up during the 5th to the 10th or 15th second, and then to fall away in rate to normal or even considerably below normal. Such a response is as follows :

> Normal at start 84 per min.
> 5th to 10th second 144 (or almost imperceptible).
> Falling away (say 20th to 25th second) to 72 or 60.

Such cardiomotor instability is frequently found in officers in need of a rest.

The results, however, apply equally well in the case of candidates (cf. Table XVIII, p. 129). In this table it will be seen that the results as a whole are in accordance with those found in the general examination, and the test is regarded as yielding valuable evidence in cases of doubt.

The value of the tests from the point of view of the examination of candidates is also illustrated by Table XVI (p. 127). In this table are seen results of the examination of (Nos. 1–13) candidates previously definitely rejected by the assessors, and (Nos. 14–18) five candidates whom the examining medical officer or the assessor 'did not like the look of'.

It will be seen that these cases are all found unfit in some respect or other by the tests employed. Further experience has fully confirmed the value of these tests in the selection of cadets.

It should be mentioned that the tests have been found useful in determining the degree of fatigue in observers coming from high flying squadrons in France for admission as pilots. Most of these

TABLE XVI

No.	Name.	Age.	Time breath held in seconds.	Vital capacity in c.cs.	Supplemental air in c.cs.	Expiratory force in mm. Hg.	Time in seconds of sustaining 40 mm. Hg by blowing.	Remarks.
1	G. E. B. ..	17 11/12	55	4,200	1,300	80	42	Rejected.
2	W. D. H. ..	17 11/12	84	4,300	1,800	60	25	Rejected.
3	J. McK. ..	18	53	3,700	1,700	55	32	Rejected.
4	H. G. R. ..	18	66	3,800	1,650	130	30	Rejected.
5	H. B. ..	17 11/12	53	2,800	1,000	60	25	Rejected.
6	R. R. S. A. ..	18	48	3,600	1,650	70	27	Rejected.
7	W. M. ..	17 11/12	44	3,400	1,600	120	35	Rejected.
8	R. C. T. ..	18	85	2,750	900	100	28	Rejected.
9	H. E. S. ..	17 11/12	71	2,400	1,050	100	50	Unfit by V.C standard.
10	F. R. ..	19	50	3,100	1,000	60	20	Rejected.
11	D. H. ..	19	63	—	—	60	40	Rejected.
12	C. T. M. H. ..	19	42	3,800	—	40	33	Rejected.
13	2/Lt. C. H. S.	23	42	4,200	—	60	25	Rejected.
14	W. A. B. ..	18 1/12	64	—	—	60	33	M.O. says fit but does not like him. Referred by assessor.
15	2/Lt. F. W. C.	22	61	4,100	—	100	30	Assessor did not like the look of him.
16	F. C. B. ..	23 2/12	63	4,300	1,800	60	35	Assessor did not like the look of him.
17	F. R. B. W.	21 6/12	55	4,100	1,700	80	37	History of Migraine referred by assessor.
18	C. T. Y. ..	18 2/12	48	3,800	1,700	100	35	History of Migraine referred by assessor.
	Average		58	3,650	1,450	77	32	

show considerable improvement after three weeks' leave, but in a certain number there is still such a degree of stress that they are not fit to go at once into training. Such subjects usually state that they have had a strenuous time and ask if it is not possible to get a ground job or a little rest. When examined with all precautions against deception by the subject, they invariably fail to pass the tests.

A few sample figures are given in Table XVII (p. 128).

It should be mentioned, however, that other candidates, although having had similar flying experience, pass the tests, again exemplifying the greater proneness to fatigue of some systems than others.

In Table XVIII are given more results on candidates taken haphazard together with the pulse response during the sustaining of 40 mm. Hg. In the ' Remarks ' column are given the assessor's findings, which were not known at the time of the examination.

E. Prognosis.

In regard to cases of flying stress it is too soon to venture an opinion as to the rate of recovery. It would seem, however, that the recuperative power of some subjects is greater than of others.

TABLE XVII

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg by blowing*	*Remarks.*
1	Lt. A. G. T. ..	23 4/12	160	13,000	5,000	62	3,600	800	90	32	Flying 7 months. Observer R.E. 8.
2	2/Lt. A. B. S. ..	21 3/12	160	22,000	17,000	50	3,000	—	110	35	Observer Bristol fighter.
3	2/Lt. H	23 4/12	120	20,000	17,000	45	3,200	1,200	70	31	Observer D.H. 4.
4	2/Lt. F.R.S.W.	24	130	22,000	18,000	35	—	—	60	30	Observer D.H. 4. Pulse 84–96 and maintained.
5	2/Lt. N. P. ..	22	105	20,000	17,000	43	3,600	1,300	60	27	Observer D.H. 4. Pulse 84–20–96.
6	2/A. M. W. B. L.	24	102	12,000	8,000	45	—	—	100	38	Observer R.E. 8. Pulse 84–32–72

TABLE XVIII

No.	Name and rank.	Expiratory force in mm. Hg.	Time in seconds of sustaining 40 mm. Hg.	Pulse before.	Pulse response.	Remarks.
1	Lt. A. B. G. ..	110	35	96	120–108 varying	160 hours observer D.H. 4.
2	Lt. H.	70	31	84	120 maintained	Unfit.
3	Lt. M	100	35	84	144–132	Strenuous time in trenches. Unfit.
4	Cadet C. ..	60	28	72	108–72	Unfit C.V.D.
5	Cadet W. ..	120	48	84	108 maintained	Fit.
6	Cadet C. ..	120	57	90	120–108	Fit.
7	Cadet S. ..	90	40	60	84–48	Unfit.
8	Cadet L. T. S. ..	130	35	90	Imperceptible 96	Unfit.
9	Lt. G. C. ..	80	41	96	144–108	Unfit.
10	2/Lt. C. T. M. H.	40	33	84	132 varying	Unfit.
11	2/Lt. H. W. L.	100	50	72	84 maintained	Fit.
12	2/Lt. R. E. R...	110	25	72	84 maintained	Malaria. Temporarily unfit.
13	Cadet E. G. M.	100	72	72	108 maintained	Fit.
14	Cadet D. H. ..	60	40	84	108–72	Unfit C.V.D.
15	Cadet D. G. P...	70	35	120	156–108	Unfit C.V.D.
16	Cadet R. W. ..	100	51	60	78 maintained	Fit. Slow intellect.
17	Lt. E. H. W. ..	60	34	96	120–72	Unfit.
18	Cadet W. A. B.	60	33	120	156–108	Referred by assessor. Found unfit.
19	2/Lt. C. H. S. ..	60	25	84	120–96	Unfit. Wounded twice. Strenuous time in trenches.
20	2/A.M. J. M. K.	120	33	120	168–72	D.H. 4 observer. Temporarily unfit.
21	2/Lt. A. B. J. ..	130	53	72	108 maintained	Fit.
22	2/Lt. F. ..	60	20	72	96 maintained	Unfit.
23	2/Lt. J. D. ..	160	54	60	84 maintained	Fit.

It will be seen that the pulse response has in many cases afforded valuable information as to the candidate's fitness, especially when taken in conjunction with other tests.

The results of some cases which have been examined two or three times are given in Table XIX.

The improvement in the time the breath is held, the vital capacity, the expiratory force, and the fatigue test is noticeable in each case (except Capt. N.). The subject also stated that he was feeling much fitter. It would seem from the case of Capt. N. that it is important that, when a certain degree of flying stress has supervened, the subject should not be allowed to fly. Capt. N., for example, begged to be allowed to continue 'limited flying', but soon found that he was not equal to it.

The results on recruits before and after one month's special respiratory exercises also indicate a great improvement in physical efficiency, an opinion vouchsafed voluntarily by the subjects con-

cerned. Such results point to the value of special exercises during the period of convalescence from flying stress, as well as in its prevention.

Prevention.

It would appear that the great hope in regard to flying stress is the prevention of its onset.

During the stage of ground training it is possible that by suitable exercises during the course of physical training, as in training for boxing or under-water swimming, the respiratory mechanism may be trained to sustain the strain imposed by flying at high altitudes. It appears that such exercises should be directed particularly to developing the expiratory force and a good tone in the abdominal wall; to accustoming the body to periods of hard work with only a limited alveolar tension of oxygen available. For this purpose, it would seem that some forms of graduated exercises with the breath held would be particularly suitable. Care would, however, have to be taken in the development of such exercises to ensure that beneficial and not harmful results were attained. This could be controlled by the application of the tests here described (see Table XIX).

When flying is begun, the systematic use of oxygen, even at relatively low altitudes, is to be recommended. (See Report No. 5 of this volume.) By a judicious hyper-oxygenation at lower altitudes the onset of deep breathing and dyspnoea is prevented at higher altitudes, and, also, the secondary ill-effects due to oxygen want. The importance of efficient administration of oxygen in this respect cannot be overstated. The fact that some pilots are more easily fatigued than others indicates that, particularly in respect of high-flying machines, the pilots should be graded with reference to their capacity for high altitude flying. Such capacity would be better estimated after their first period of training. It is probable that many pilots who break down from stress on scout machines would have rendered a much longer period of service on lower flying machines. During this time, however, there is no doubt that physical exercises, judiciously employed, will also serve to avert the onset of stress.

In regard to pilots who break down early on scout machines, it would be advisable if, before being stopped from flying altogether, they were medically examined, with a view to testing their capacity for flying at lower altitudes.

During the period of training in the air the pilot should be carefully watched. It is perhaps not too much to say that with careful medical overhaul, an efficient body of delivery pilots might be salved from officers who at present are being deemed unfit for further instruction in flying altogether. This is especially true of pupils who are found unfit to fly high altitude machines.

An experiment in this direction was made at a special medical flight, with the result that quite a number of pilots were 'salved', some of whom afterwards rendered service overseas. These officers were all selected from pupils who had been considered unfit for further instruction in aviation.

In the preliminary overhaul of these officers by the tests described in this paper, the interesting fact emerged that most officers who were found to be temperamentally unfit to fly were (in many cases) found to be physically unfit, whereas the officers who were keen to continue flying were found to be fit from the standard of these tests. In all over 300 such officers were examined. A sample selection of these examinations is given in Table XV.

No. 13 in the table below, although found physically fit, was certainly temperamentally unfit. It was ascertained later that he was a truculent, cross-grained fellow, undisciplined, and caused a lot of trouble throughout his career. Another pupil (No. 15) had developed 'anxiety'. He was not examined by a neurologist but was probably a psychopath. He always experienced great anxiety while in the air and was certainly unfit to continue flying and definitely wished to give it up.

It is difficult to say whether in other cases the unfitness was induced by unsuitable work or whether the candidates were unfit for flying from the outset. Probably they were mostly fit subjects who reacted badly to the strain of flying.

The above results, however, indicate that the flying temperament in many cases goes hand in hand with physical fitness. In most cases it is only when the officer is physically unfit that the opinion is formed that he is unfit for flying, or he himself comes to the conclusion that he is unfit for flying and wishes to cease altogether.

F. Treatment.

In regard to treatment of cases broken down through flying stress, it is probable that the judicious employment of physical exercise may produce beneficial results. (See Table XIX.) Violent forms of exercise should be avoided, but riding and walking are to be advocated since they call for the expenditure of little nervous energy. Where physical exercises are employed, care must be taken that these are producing beneficial results. Help in this direction may be afforded by the tests herein set forth.

The treatment of a series of cases by oxygen inhalations in a special chamber similar to that employed in 'gas' cases would be of interest.

G. Discussion of Results.

In a preliminary paper such as this, it is not intended to attempt full explanation of the results obtained. The need of fuller information is great on so many points that the most that can be done is to discuss the results in a tentative fashion.

The results so far obtained indicate that when flying stress has markedly supervened, the power to hold the breath, vital capacity, supplemental air, expiratory force, and the power to sustain 40 mm. Hg by blowing, are all decidedly lessened. It also appears that the most delicate of these tests is that of attempting to sustain 40 mm. Hg pressure by blowing with the breath held. By this test it has been found possible to grade pilots according to their capacity for flying, a capacity adjudicated not by the writer but by others, such as the

TABLE XIX

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg.*	*Remarks.*
1	2/Lt. H.E.K.G.	19 6/12	2 hrs.	2,500	700	74	3,000	—	60	12	C.V.D. Fainted at 650 ft.
			40 m.			69	3,400	—	100	40	After 2 months Convalescent Home.
2	Lt. E. M. G. ..	26	150 *o*	16,500	5,000	37	3,600	750	45	23	Stood off by Medical Officer.
			150 *p*			55	3,700	1,000	60	23	Fortnight later.
						95	4,000	1,400	100	52	Fortnight later.
3	Capt. C. S. T. L.	21 6/12	125 *o*	22,000	13,000	63	3,550	1,600	125	23	Stood off by Medical Officer.
			200 *p*			83	4,400	1,600	140	45	2 months later.
4	Capt. R. G. N.	22 11/12	1,500	23,000	10,000	40	4,100	1,750			
						42	4,100	1,700	60	28	—
									60	25	6 weeks later, after limited flying.
5	Capt. W. R. B.	25	700	17,500	15,000	72	3,900	—	110	43	Fatigue.
						80	4,480	—	170	58	? C_2H_5OH. Three weeks after 'taking care' of himself.
6	Lt. H. A. J. ..	24	86	20,000	15,000	55	4,150	—	120	35	? Auto-intoxication.
						80	4,560	—	130	46	10 days later, after giving up smoking and alcohol.

		Pulse : Standing.	*After exercise.*	*Return to normal.*					
7	Bo.	96	120	30	—	3,580	—	86	22
		84	96	15	—	3,790	—	128	37
8	Br.	108	132	20	—	3,480		90	38
		96	108	15	—	4,000	—	114	64
9	Co.	96	120	40	—	4,080	—	78	22
		72	96	20	—	4,520		88	47
10	Re.	84	108	25	—	3,980	—	72	38
		81	108	20	—	4,080	—	88	55
11	McI.	96	108	15	—	3,400	—	120	41
		60	72	15	—	3,360	—	155	44

Recruits before and after one month's special respiratory exercises. Upper line before, lower line after, training.

Recruits before and after one month's special respiratory exercises. Upper line before, lower line after, training.

o = Observer. *p* = Pilot.

TABLE XX

No.	*Name and rank.*	*Age.*	*No. of hours flying.*	*Maximum height.*	*Average height.*	*Time breath held in secs.*	*Vital capacity in c.cs.*	*Supplemental air in c.cs.*	*Expiratory force in mm. Hg.*	*Time in secs. of supporting 40 mm. Hg. by blowing.*	*Pulse response.*	*Remarks.*
1	2/Lt. C. P. A. ..	21 8/12	6 *d* 2 *s*	3,000	1,500	30	3,400	—	80	25	96–132–108	No question of wishing to continue to fly.
2	2/Lt. R. H. ..	28 3/12	10 *d* 4 *s*	18,000	5,000	62	4,100	—	130	43	96- 132 and maintained	Keen to fly. Doing well at Hendon. Medical flight.
3	2/Lt. C. G. T. ..	19 8/12	5 *d*	5,000	1,500	30	3,000	—	60	18	108–inappreciable–72	Does not wish to go on. Feels he is a 'dud'.
4	2/Lt. J. A. B. ...	19	35	15,000	6,000	66	3,500	—	100	53	108–120–108	Keen to go on flying.
5	2/Lt. H. D. O'D.	23 6/12	5 *d* 5 *s*	5,000	2,000	10	2,900	—	40	8	72, pulse went thready and could not be counted	Has suffered from colitis. No wish to fly at present.
6	2/Lt. K. L. G. ..	24	5 *d* 10m *s*		—	50	3,100	—	100	40	72–84–72	Very keen to go on. Now at medical flight.
7	2/Lt. J. M. S. ..	32	—	2,000	—	32	—	—	70	20	84–132	One crash. No wish to go on flying.
8	2/Lt. H. D. L. ..	19	40	14,000	8,000	15	—	—	40	8	84 and inappreciable	No wish to go on. History of crashes and bad landings.
9	2/Lt. W. J. K...	19	5 *d* 8 *s*	10,000	3,000	29	2,900	—	60	12	—	Headaches after flying. No wish to continue.
10	2/Lt. A. C. M. ..	19 2/12	3 *d*	—	—	66	—	—	120	48	84–108	Keen to go on. Now at Hendon. Medical flight.
11	2/Lt. D. B. B. ..	18 6/12	5½ *d*	—	—	49	3,100	—	100	40	Fairly good	Keen to go on. Now at Hendon. Medical flight.
12	P/Fl. O J. G. J.	18	4½ *d* 10 *s*	3,000	2,000	68	4,100	—	70	35	60–96–60	Crashed. No wish to fly again. Tremor eyelids and fingers.

13	2/Lt. J. S. McC.	18 2/12	—	—	—	—		—	80	60	60–72 and maintained	Crashed 4th solo. Temperamentally unfit. Caused lot of trouble always.
14	2/Lt. H. D. W.	19 3/12	2½ *d*	—	—	—		—	60	25	Pulse becomes impalpable	Temperamentally unfit.
15	C. N. S.	18 4/12	7.53 *d*	7,000	6–700	61	—	—	160	52	84–96–72	Temperamentally unfit; fit physically.
			1.43 *s*	—	—	—	—	—	—	—	—	
16	W. R. R.	27 7/12	6.30 *d*	—	—	—	—	—	—	—	—	Very keen.
			1.31 *s*	6,200	1,000	57	—	—	100	43	108–132	
17	J. W. P.	22 11/12	7.47 *d*	—	—	—	—	—	—	—	—	Temperamentally unfit.
			0.52 *s*	3,500	1,000	32	—	—	100	22	80–108	
18	A. E. R.	26 7/12	7.4 *d*	4,000	1,000	35	—	—	75	28	72–108	Temperamentally and physically unfit.
			2.36 *s*	—	—	—	—	—	—	—	—	
19	C. G. B.	23	5 *d*	—	—	68	—	—	100	43	101–132–120	Keen to continue flying
20	B. W. N.	19 4/12	13 *d*	—	—	—	—	—	—	—	—	Temperamentally unfit, physically unfit.
			2 *s*	—	—	35	—	—	75	20	66–132–106	
21	R. E. Y.	19	3 *d*	—	—	26	—	—	70	18	80–108–96	Temperamentally unfit, physically unfit.
			4½ *s*	—	—	—	—	—	—	—	—	
22	E. McN. H. ..	29 9/12	7 *d*	—	—	24	—	—	70	17	108–144–132	Temperamentally unfit, physically unfit.
			½ *s*	—	—	—	—	—	—	—	—	

d = Dual. *s* = Solo.

squadron or flight commander, the aerodrome medical officer, or the Invaliding Medical Board.

The following explanation of flying stress is here put forward tentatively. It develops to the greatest extent in high flyers and is of gradual onset. The rate of this onset appears to be closely associated with the respiratory capacity and expiratory force of the individual, being slower in those well developed than in those in these respects less well developed.

A good respiratory capacity is necessary for the efficient intake of the rarefied air at altitudes, a good expiratory force for breaking out against the slip stream of the propeller and the contrary wind created by the rapid progress of the machine through the air. It is calculated that the force of this wind in a machine travelling at 100–120 miles an hour may on occasion amount to 60 to 80 mm. Hg, showing how necessary is a good expiratory force.

During an ascent the respiratory system is the first affected, owing to the lessened oxygenation of the blood in the rarer air, so that a hyperpnoea of gradually increasing intensity is developed. With increasing height all the accessory respiratory mechanism is called into play, especially with the onset of definite dyspnoea. At high altitudes, also, periodic breathing (Cheyne-Stokes) is not uncommon. At this stage there is frequently definite evidence of oxygen want, as manifested by the ashen face and blueness of lips.

The pulse is greatly quickened, nervous symptoms, subjective or objective, may be present (see Lieut.-Col. Birley's Report No. 1 in this volume). At this stage the strain thrown upon the various systems is exceptionally great, particularly so upon the bulbar centres regulating the respiration and the circulation.

It is suggested that when, as in high flyers, this strain is frequently repeated, these centres, particularly the respiratory centre, become affected in such a manner that the regulation of respiration is no longer normal.

In regard to the respiration, the power adequately to ventilate the system is reduced owing to loss of expiratory force, breathing becomes shallower, lessened oxygenation of the blood follows, bringing in its train secondary circulatory and nervous symptoms. This agrees with the results observed by Haldane, Meakins, and Priestley on 'gassed' cases.[1] (See also report by Corbett and Bazett in this volume, p. 18.)

As regards the circulatory system, there may result eventually a tachycardia or disordered action of the heart, due most probably to an altered condition of excitability of the cardio-motor centre. This is well shown in many cases by the characteristic response of the pulse during the test of sustaining 40 mm. Hg by blowing with the breath held.

As has been shown also with the onset of flying stress, the pulse pressure gradually increases, a condition due, it is believed, to a lax tone of the arterioles, resulting possibly from an altered state of stability of the vaso-constrictor centre. The altered state of tone of the vaso-dilator centre is exemplified by the marked, almost instan-

[1] Haldane, Meakins, and Priestley, *Journ. Physiol.*, 1919, lii, p. 420.

taneous flushing of the face, which follows in the fatigued subject on the smallest effort at blowing.

In respect of the suggestion that the respiratory centre fails first, it is interesting to note that Professor W. M. Bayliss has recently found in his work on shock that if an animal be left with a low blood pressure after the removal of a large quantity of blood, the medullary centres become insensitive to stimulation. When the blood pressure is raised and artificial ventilation continued, the vaso-motor centre may recover, but the respiratory centre does not recover more than to give three breaths a minute. (Personal communication.)

Although attention is here directed particularly to the bulbar centres, it is to be noted, however, that owing to 'anoxaemia' there is usually an effect upon the central nervous system generally. It is at this stage the pilot feels, for some reason he cannot explain, a disinclination to fly. He feels 'dud', 'sick of the air', 'hates the sight of a machine'. Sleep may be bad or he may often experience nightmares. He is generally 'flying-tired', and in this connexion the loss of expiratory force is illuminating.

Although a loss of power is perhaps first associated with the respiratory mechanism, especially with the abdominal muscles concerned in expiration, there is frequently at this stage a loss of muscular tone generally.

When the stage of breakdown is reached, the subject has to give up flying altogether, and at this stage the cardio-vascular psychic or nervous symptoms become more marked, as does also the general loss of muscular tone.

In regard to the voluntary system, this loss is shown by a lessened force of the cheek and mouth muscles; in respect of the involuntary system by the loss of tone in the stomach and bladder walls.

At this stage it is difficult to forecast whether a case will be regarded as primarily nervous, e. g. nervous breakdown, nervous exhaustion, neurasthenia, and be passed to the neurologist, psychologist, or as primarily cardio-vascular (cardio-vascular debility) and passed to the physician.

It is suggested that an abnormal state of the bulbar centres with defective respiratory ventilation may be found in cases of stress other than flying. Professor Dreyer[1] has recently shown that the vital capacity of a subject can be accurately calculated from the weight, stem length, and chest circumference of the individual. The exact diminution of the vital capacity in a subject can therefore be accurately ascertained, and it is probable that this measurement as well as the test for expiratory force and the test of sustaining 40 mm. Hg by blowing would indicate the degree of stress in subjects after a long and trying period in the trenches. This has been found to be true in several cases examined. It would be of interest, also, to apply the test among subjects liable to suffer from industrial fatigue, such as munition workers.

[1] Dreyer, loc. cit. (p. 95).

H. Conclusions.

1. The measurement of the vital capacity is important in candidates for aviation and in pilots. Its adequacy should be calculated from the formulae put forward by Dreyer. The vital capacity is greatly diminished in cases of flying fatigue. In its measurement the subject sits opposite the spirometer, the dial being so placed that he cannot see the readings. Movement of neck, chest, and abdomen must be unhampered. He is directed to fill his lungs to the fullest extent and then to expire as completely as possible through the meter. Five readings should be taken.

2. Other simple tests are described which it is believed will prove valuable in ' assessing ' flying stress and also in determining the fitness of candidates for flying. These tests should be used in conjunction with the points found in the medical examination.

3. The test for breath-holding capacity is as follows :

The subject is asked to expire as deeply as possible (preferably audibly), and then to fill the lungs fully (but not to absolute distension), and to hold the breath with the nose clipped or held. The reason for the audible expiration is that it has been observed that some subjects suffering from stress have lost the power of expiring fully, and a marked shortening of time taken to expire, as fully as possible, may afford an indication of such loss of power. The time during which the breath is held is noted, and the subject is then asked the reason for giving up. This should be recorded. The significance of the test and the time that it is expected must not be communicated, and on no account may any one time himself.

The subject should be asked the reason for ceasing. Normally, an answer such as ' I had to give up ', ' I felt I should burst', ' I wanted to breathe ' is given. Subjects suffering from marked disability at altitudes almost invariably return an abnormal answer, e. g. ' I became giddy or dizzy ', ' Things went blurred ', ' The blood rushed to my temples ', ' I began to feel squeamish '. Where the answer is deemed not to be normal the subject's own words should be given.

The average time the breath is held by the normal fit pilot is 69 seconds, the minimum time being 45 seconds. Generally speaking, a man who does not hold his breath 45 seconds should not be admitted as a pilot. As a matter of experience it will be found that very nearly all such cases will be rejected on medical grounds, apart from this test. The test is believed to afford indication of

(*a*) The stability of the respiratory centre, and indirectly, of the nervous system generally ;
(*b*) The likelihood (when the time is short and an abnormal answer is given) of the subject suffering from oxygen want at altitudes ;
(*c*) Resolution to ' carry on ' under conditions of stress.

4. The test for Expiratory Force is carried out in the following manner :

The applicant or airman is asked to hold his cheeks with the thumb and forefinger of the left hand, and steadily blow the mercury

column of the standard U-tube up as high as possible with the scale turned away. Several mouth-pieces should be kept in disinfectant and carefully cleaned for each subject. On no account must the applicant or airman be allowed to swing the mercury up violently, and in all cases the height of any initial swing is to be disregarded. The reason for holding the cheeks is that it has been found that in some cases the mercury may be forced up to abnormal heights by the action of the cheek muscles. The number of mm. Hg blown is recorded. The examinee is then asked to repeat the performance while looking at the column. The resolute subject may, under these conditions, considerably pass the effort which he did not see, in this way affording some indication of his mental make-up. On the other hand, a subject who is not trying may give very discrepant readings. With the manometer again turned away, he may, by encouragement, be made to surpass his previous efforts, but when, after trying again whilst looking at the manometer scale, he will not surpass if he is not really trying the previous effort which he saw.

The average for normal individuals is about 105 mm. Hg. When under 80 mm. Hg it suggests that the subject will probably be incapable of sustained effort in routine aerial work, and should probably be rejected. The test, however, should be taken in conjunction with the results of the rest of the examination.

5. The test of sustaining 40 mm. Hg with the breath held is performed as follows :

The subject is asked to empty the lungs, fill up, blow the mercury to the height of 40 mm., and hold it there *without breathing* for as long as possible, preferably with the nose clipped. The average normal time in the large number of cases tested is 50 seconds. Below 40 seconds is unsatisfactory, and indicates that probably the subject is fit only for limited flying or in need of a rest. This can be assessed from the other points noted during the examination. Pilots broken down from stress of service have an average of about 25 seconds.

6. A valuable adjunct to the test is the behaviour of the pulse during the time the mercury is being sustained. This is counted during each five seconds that the mercury is sustained. Starting at the 5th second in the normal individual, there is generally a steady slow rise in the rate of the pulse, or a fairly marked rise, which is sustained most of the time. For example, the pulse rate may rise gradually from 72 to 96 or 108, according to the time the breath is held, or the pulse may rise almost at once from 72 to 98 or 108, and be sustained there. A large rise in rate, e. g. from 72 to 132 or 144, is unsatisfactory. In cases of flying stress the characteristic response is for the pulse to jump quickly up during the 5th to the 10th or 15th second, and then to fall away in rate to normal or even below normal. A bad response is as follows :

Normal at start, 84 ;
5th–10th second, 144, often almost imperceptible ;
Falling away (say 20–25th second) to 72 or even 60.

7. The above tests show the necessity of a firm-toned abdominal wall in the flying officer. This is of importance for the maintenance

of efficient respiratory ventilation, and also to prevent any tendency to the pooling of blood in the abdomen.

8. The fact that the first indications of ' fatigue ' are associated with the loss of power to sustain 40 mm. Hg, and also frequently with a diminished capacity to hold the breath, suggests the tentative explanation that the first stage in the onset of flying fatigue is due in many cases to an altered tone of the respiratory centre. This leads later to a diminution of the vital capacity, supplemental air, and loss of expiratory force. Defective respiratory ventilation then brings about lessened oxygenation of the body, which reacts upon other systems, particularly upon the circulatory and nervous systems.

8.—TEMPERAMENT AND SERVICE FLYING

BY

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The object of this paper is first to draw attention to certain aspects of service flying with which medical officers in the Field are confronted every day, and secondly to discuss the significance of certain of the tests introduced by Lieut.-Colonel Flack and the results obtained by their employment (*vide* No. 3 of this volume).

A pupil under instruction is chiefly of interest from the point of view of his capacity as a pilot (i. e. a chauffeur), whereas in the Field the fully-fledged flying officer absorbs our attention in his capacity as a combatant; in fact, it cannot be too often insisted that an aeroplane is *par excellence* a gun mounting, designed to inflict losses on the enemy in his own territory. It therefore follows that the flying temperament, i. e. the temperament for flying *per se*, is of little importance in the Field, since those devoid of it do not, with few exceptions, reach the active service stage. The temperament for fighting and for combatant service in general is, on the other hand, a matter demanding our close attention.

It is not within the ability of the writer to attempt any close analysis of what is known as Temperament. Suffice it to say that it is intimately bound up with Character, both hereditary and acquired, and plays a large part in determining an individual's conduct and manner of life. The War has created a situation in which hundreds of thousands of men have been required and expected to expose themselves to discomforts and dangers undreamt of four years ago, and it is mainly owing to fundamental differences in temperament that the requirements have not been, and never can be, equally fulfilled. Equality of Service is a most desirable object to aim at; it is none the less desirable to recognize that it is impossible of attainment. It accordingly happens that we constantly meet with individuals who are incapable of sustaining for an average period the effort, physical and mental, which service as a combatant demands. This is true of all branches of the Service, but each branch demonstrates its own peculiarities. The fundamental peculiarity of combatant service in the air lies not so much in the fact that the air is not the natural element of Man, but rather in the circumstance that flying demands and encourages a degree of individualism unknown in any other branch of the Service. Formation flying, no matter to what extent it is developed, although it can diminish, can never entirely abolish, this outstanding peculiarity, which is a hall-mark of the Air Service.

These considerations attain considerable practical importance in dealing with individuals who, on their own showing or in the opinion of their Commanding Officers, are temperamentally unsuited for service flying. Such an individual may not only be fond of flying, but may even be a skilled pilot. Even so, is it proper to turn him

into an instructor, a test pilot, or a ferry pilot ? As an instructor, with but few exceptions, clearly No, lest his temperament infect his pupils ; for it must be remembered that he will be instructing others to fly in order that they may eventually fight. As a test or ferry pilot ? In the opinion of the writer the answer depends entirely on the degree of physical fitness of the individual in question. If he is not fit for combatant service on the ground he will be clearly most usefully and economically employed in non-combatant service in the air. If fit on the ground, however, the decision rests entirely with the executive authorities, who may decide to transfer him to the infantry. It may be asked, Why if unfit for temperamental reasons for combatant service in the air can an individual be considered likely to prove of value as a combatant in the trenches ? The answer has already been discussed. On the ground fewer calls will be made on his individuality, and more opportunities will be afforded for gaining confidence in himself from intimate comradeship with those of tougher calibre, with the result that he may eventually discover himself, and even acquire the art and spirit of leadership.

The term temperamental unfitness has crept into use largely for want of a better one, and its precise meaning is a source of frequent discussion and considerable perplexity. It might be said, for example, that a successful pilot suffering from stress of service was temperamentally unfit. This view is incorrect ; he is unfit only in so far as his temperament, i.e. his outlook on things and powers of resistance, has undergone modification as the result of fatigue, whereas he is clearly not unfit by virtue of his temperament regarded as part of his normal ego. Youth and immaturity are also factors to be taken into consideration (*vide* Table VII, case 8).

It is now recognized that Temperament is a matter for study by executive and medical officers in conjunction, since the line which divides the physically from the temperamentally unfit is, as will be presently pointed out, as imaginary as the equator.

Before proceeding to analyse the results of the physical examination of different categories of pilots, it will be as well to say a few words on the methods employed. Nearly all the cases have been examined by Captain C. D. H. Corbett and Captain H. C. Bazett, M.C., to whose unfailing industry I should like to pay a grateful, if wholly inadequate, tribute.

Every patient brings with him a brief history of his case made out by his Wing Medical Officer, and in most instances a few lines from his Squadron or Flight Commander. He is examined the morning after admission, and is not allowed out of bed until his turn for examination comes. He then walks into the examining room in dressing-gown and slippers, and sits down.

The routine examination is conducted as follows :

1. Past medical history and military history, i.e. previous service abroad, whether combatant or not, date of joining the Air Service, by whom medically examined, date of wings, date of qualifying as observer, number of hours flown in England and on active service, type of work, &c.
2. Age, height, weight.

3. Present history leading up to his admission to hospital.

By this time the examiner should not only have obtained some valuable information, but should also have gained the confidence of his patient, and all nervous excitement due to the immediate surroundings should be in abeyance.

The physical examination is then conducted as follows :

4. The pulse is counted over one minute and recorded every 15 seconds.

   a, sitting ; *b*, standing ; *c*, after exercise, which consists in the patient raising himself slowly and in the upright position five times on to a chair.

5. Examination of the heart and lungs.
6. Determination of the systolic and diastolic blood-pressures by the auscultatory method.
7. Expiratory force, as determined by the manometer.
8. Time in seconds of holding up the mercury to 40 mm., and the behaviour of the pulse during the test.
9. Respiratory rate at rest.
10. Vital capacity, and tidal air.
11. Ventilation per minute at rest.
12. Examination of the abdomen and abdominal wall.
13. Condition of the reflexes ; presence or absence of tremor.
14. Condition of the tympanic membranes.
15. Presence or absence of Eustachian obstruction.
16. Visual acuity.

In addition, the writer makes it a practice of having private consultations, lasting five to ten minutes, with selected patients. These are made as unofficial as possible, and frankness of utterance is encouraged on both sides.

In the following tables the results only of the pulse rates, blood-pressures, expiratory force, and sustaining the mercury test are recorded. As regards the two former no attempt at close analysis is made, still less is any effort made to discuss the results from the point of view of the mechanical efficiency of the cardio-vascular system. It has been shown by Starling and his co-workers by the use of the heart-lung preparation that the heart is capable of responding within wide limits to changes in the mechanical demands made upon it, so that ' it is difficult to imagine a more perfectly regulated machine than the heart '. How wide these limits can be is demonstrated in the following tables, where high and low blood-pressures are combined with fast and slow pulses in a seemingly random manner.

Flack has tentatively put forward the hypothesis that the first stage in the onset of flying stress, especially in flying at high altitudes, is an altered condition of tone of the respiratory centre of such a kind that the regulation of respiration is no longer normal, and that this alteration is demonstrable by the ' sustaining the mercury test '. In view of this hypothesis it has been thought advisable to give the results obtained in a series of pilots and observers who have been recommended by their commanding officers for transfer to England for a rest on account of fatigue. The results will be found in Table I.

TABLE I. PILOTS AND OBSERVERS TRANSFERRED TO HOME ESTABLISHMENT FOR A REST

(*a*) BRISTOL FIGHTER AND D.H. PILOTS AND OBSERVERS

Serial Number, Rank, and Initials.	*Age.*	*Remarks.*	*Months since posted Overseas.*	*No. of Hours War Flying.*	*Expiratory Force in mm. Hg.*	*Time in Seconds of Sustaining 40 mm. Hg by Blowing.*	*Blood Pressure.*	PULSE. *Sitting. Standing. Exercise.*	*Remarks.*
1. Capt. R. N. C. A., M.C.	21	Long spell overseas. 1916–17. Pilot.	2	420 (1916–17) 80 (1918)	140	40	105/70	72 90 90	Pain in ear due to Eustachian obstruction. Very hard work lately. Feels very tired. Returned one month later. Doing very well.
2. Capt. C. P. O. B., D.S.C.	29	Very experienced pilot Long bombing.	18	300	90	42	102/67	73 102 109	Not slept well for one year. Feels done up. Headache on landing. Short of breath on ground.
3. 2nd Lt. W. D. B.	26	Pilot.	6½	170	—	—	125/75	74 83 97	Headache on landing. Feels slack and tired. Loss of interest.
4. Capt. B. E. B., D.S.O., M.C.	21	550 hours as instructor. Has done much long-distance running.	9	150 (1916) 300	180	57	120/85	80 78 85	Never affected by altitude until recently. Now severe landing headache. Otherwise fit. Lost some of his dash.
5. Capt. D. G. C., M.C.	22	Pilot. Flight Commander.	7	210	120	48	136/85	71 85 94	Not affected by altitude, but now tired after flying. Losing his dash.
6. Lt. H. G. C., M.C.	20	Observer. Non-smoker. Teetotaller.	5	144	190	48	135/95	54 64 67	Feels fit and ready to learn to fly.

7. Lt. T. G. J.	20	Pilot.	7	150	90	48	115/90	88 103 107	Fainted recently at 13,000 feet. Feels tired and washed out.
8. Lt. S. H. P. M.	21	Pilot.	6½	160	100	47	135/98	76 82 99	Not as happy over the lines as he used to be.
9. H. F. M.	20	Pilot.	6	120	165	59	112/75	77 89 101	Feels tired ; cannot stand heights nearly as well as he could.
10. Lt. L. H. P.	23	Pilot.	6	160	145	61	108/68	63 71 71	Very heavy work lately, and is feeling the effects.
11. R. S.	22	N.C.O. Observer;	12	150	120	40	140/70	82 89 100	'Wind up' one month—quite fit before. Doesn't want to learn to fly at present.
12. Capt. E. I. S. J.	20	Pil t. Flight Commander.	7	215	72	30	112/70	63 73 80	Latterly had to force himself into the air. Cannot stand heights now, gets so short of breath.
13. 2nd Lt. A. T. H. T.	19	Observer. Rejected by C.B. as pilot	5	100	180	45	120/75	76 92 99	A bit shaky lately. No leave. Very keen to learn to fly. Used to be long-distance runner.
14. Capt. V. S. W.	21	Pilot. Flight Commander.	15	160	90	48	134/72	80 102 120	'Hopelessly stale.'
15. Lt. C. D. T.	21	Pilot.	3	150	100	46	135/80	72 83 95	Very hard work lately. Looks and feels tired out. Headache on landing.
16. Capt. L A. P., M.C.	24	Pilot. Flight Commander.	9	234	150	35	110/75	63 69 81	Feeling fit except for dental trouble. Still unaffected by altitude.

TABLE I. PILOTS AND OBSERVERS TRANSFERRED TO HOME ESTABLISHMENT FOR A REST

(*a*) BRISTOL FIGHTER AND D.H. PILOTS AND OBSERVERS (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Remarks*	*Months since posted Overseas.*	*No. of Hours War Flying.*	*Expiratory Force in mm. Hg.*	*Time in Seconds of Sustaining 40 mm. Hg by Blowing.*	*Blood Pressure.*	Pulse. *Sitting. Standing. Exercise.*	*Remarks.*
17. Lt. E L.	21	Pilot. Teetotaller.	7½	260	110	43	125/70	63 79 99	Worried by A.A. shells, which he never used to be. Feels slack.
18. 2nd Lt. G. D.	26	Observer. Rejected by C.B. as pilot.	8	140	198	60	140/100	83 87 90	Magnificent physique. Sick of observing. Wants to learn to fly.
19. Lt. C. D. P.	21	Observer.	7	160	90	65	118/74	51 76 87	Highly strung. Sleeping in air lately. Feels fairly fit, but stale.
20. Lt. S. A. O., M.C.	27	Pilot.	7	180	150	82	114/72	70 84 89	Entirely unaffected by altitude. Tired and stale. Lost keenness.
				AVERAGE	130·5	49·7	121/74	71 84 93	

(*b*) SCOUT PILOTS

1. Capt. J. O. A., D.S.O., M.C.	21	200 hours as an instructor. Seen a lot of service.	1	600 (1915–17) 40	70	60	122/85	63 89 91	Not fit when he came out. Sleeping badly two years. Depressed, muddle-headed. Feels strain of war flying.
2. Capt. F. B.	23	200 hours H.D, Flight Commander.	3	300 obs 200 (1917) 90	90	40	135/80	81 89 97	Not fit when he came out. Vomiting in air, getting worse. Headache, general fatigue. One month later returned to night bombers. Since awarded D.F.C.

3. Capt. L. D. B.	29	Flight Commander.	9	200 obs. (1916) 275	104	46	122/78	60 68 80	Tired-reaction after flying. Much low strafing lately. 6 Dioptres of Myopia in each eye.
4. Capt. H. H. B., M.C.	20	Flight Commander. Excitable type.	4	170 (1917) 150	86	27	130/72	69 77 86	Low strafing lately. Absolutely worn out. Dreams every night that he is taken prisoner.
5. Lt. C. F. C.	28		5	125	82	61	95/70	61 76 71	Nearly fainted in air short while ago. Otherwise feels fit.
6. Capt. H. F. S. D., M.C.	23	Flight Commander.	8	250	130	60	106/66	63 89 91	Very tired—chiefly result of low strafing.
7. Capt. S. E., D.S.C.	25	Flight Commander.	24	450	120	43	115/90	68 97 109	Feeling done in last month. Broke down yesterday after patrol.
8. Capt. O. H., M.C.	25	Flight Commander. 3 times wounded in infantry.	6	190	90	60	135/90	59 75 81	Sleeping badly lately. Anginal attack in air recently. Killed flying in England 19.8.18.
9. Lt. R. G. H., M.C.	20		8	250	112	57	120/90	83 90 104	Feels fit but gets praecordial pain at high altitudes and progressively tired after landing.
10. Lt. T. F. L.	26	a/Flight Commander.	7	150	90	40	135/95	86 94 98	Not sleeping well. Feels 'done up'. Kept going on C_2H_5OH.
11. Capt. K. M. L., M.C.	21	Flight Commander.	7	150 (1916) 200	125	42	104/60	61 69 76	Tired one month. Loss of interest. Persistent headache.
12. Capt. G. E. H. McE., M.C., D.F.C.	24	Flight Commander. Asthmatical, some emphysema.	8	215	110	45	115/82	66 79 80	Very tired—low strafing. Returned two months later and did magnificently. Reported missing (1.8.18).

TABLE I. PILOTS AND OBSERVERS TRANSFERRED TO HOME ESTABLISHMENT FOR A REST

(*b*) SCOUT PILOTS (*continued*)

Serial Number, Rank, and Initials.	*Age*	*Remarks.*	*Months since posted Overseas.*	*No. of Hours War Flying.*	*Expiratory Force in mm. Hg.*	*Time in Seconds of Sustaining 40 mm. Hg by Blowing.*	*Blood Pressure.*	PULSE. *Sitting. Standing. Exercise.*	*Remarks.*
13. Capt. H. H. R., M.C.	21	230 hours testing Flight Commander.	4	140	130	60	140/85	67 83 86	Drowsy in the air. Getting jumpy and losing confidence. Recommended for Australian leave.
14. Lt. C. L. S.	18	Very small physique.	6	220	120	25	115/90	94 102 117	Excitable type. Unaffected by altitude. Feels fit.
15. Lt. H. W. S.	21	Observer (1916–17).	5	160 obs. 120	120	40	120/76	83 104 102	Nearly fainted in air two days ago. Feels tired and slack.
16. Capt. W. B. T.	25	Flight Commander.	5	170	150	45	140/98	62 85 94	Getting jumpy. Goes to sleep in the air and feels incapable of looking after his flight.
17. 2nd Lt. C. T. T.	19	100 hours instructing.	7	250	120	63	102/62	63 77 84	Headache last three weeks. Otherwise fit. Looks tired.
18. Lt. H. S. W.	19	Diminutive physique.	8	170	98	22	140/85	92 104 110	Badly shot about lately. Has lost confidence.
19. Lt. P. E. W.	29		9	200	120	64	130/90	71 86 99	Been making bad landings. Feels thoroughly tired. Nightmares.
20. Capt. T. E. W.	26	Flight Commander. Observer (1916–17).	7	185 obs. 195	85	55	120/80	54 64 76	Never sick since war began. Latterly been less successful in air. Feels fit.

21. Lt. H. L.	19		4½	160	120	45	115/75	84 100 106	Lost his dash. Looks and feels tired.
22. Lt. P. W.	19		5	173	120	35	140/100	68 72 84	Not flying so well lately. Lost dash and energy.
23. Capt. A. W. C., D.S.C.	24	Flight Commander.	15	500	100	60	122/80	64 78 81	Very stale—lost the offensive spirit.
24. Lt. K. G. P. H	19		6	170	170	60	138/70	—	Crashed 14 days ago—unconscious three days. Now feels 'full out'.
25. Capt. C. O. R.	22	Flight Commander.	7	200	160	75	96/58	60 70 75	Feeling strained. Sleepy above 14,000 feet. Praecordial pain on ground.
26. Capt. G. C. M.	34	Flight Commander.	5	150	130	43	110/60	74 79 91	Worried about defective vision in one eye when leading patrols. Otherwise fit.
				AVERAGE	108·5	47·2	121/80	70 83 90	
				(c) ARTILLERY PILOTS AND OBSERVERS					
1 Lt. W. A. M.	20	Observer.	6½	180	205	53	122/88	68 79 91	Recent perforation of drum. Feels fit. Very keen to become pilot.
2. Lt. J. E. A.	23	Pilot.	6	150	100	31	110/75	65 83 96	Bad landings last ten days. Nearly fainted in air. Some loss of confidence.

TABLE I. PILOTS AND OBSERVERS TRANSFERRED TO HOME ESTABLISHMENT FOR A REST

(*c*) ARTILLERY PILOTS AND OBSERVERS (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Remarks.*	*Months since posted Overseas.*	*No. of Hours War Flying.*	*Expiratory Force in mm. Hg.*	*Time in Seconds of Sustaining 40 mm. Hg by Blowing.*	*Blood Pressure*	PULSE. *Sitting. Standing. Exercise.*	*Remarks.*
3. Lt. W. J. A.	21	Observer in 1916–17.	5	200 obs. 200	105	35	140/85	66 88 99	Fainted at 6,000 feet six weeks ago. Headache. Feels he has had enough.
4. Lt. C. E. A.	20	Pilot.	9	200	180	30	142/105	68 86 100	Not the least bit tired or strained.
5. Lt. H. A. B.	21	Pilot.	8½	200	170	48	140/95	61 68 83	Lost interest in his work. Feels well in himself.
6. Lt. S. H. B.	27	Observer.	4	190	90	40	105/70	73 87 90	Praecordial pain three weeks. Tired—has lost confidence.
7. Lt. W. M. E.	21	Pilot.	8	160	170	55	115/75	66 92 95	Completely tired out. No energy left.
8 Capt. J. S. G., M.C.	23	Flight Commander.	13	330	100	32	130/95	65 82 104	Loss of confidence in air one month. Restless.
9. Lt. R. C. H.	24	Observer.	8	210	108	70	124/75	73 78 73	Restless. Eyes tired—standard of work has fallen off.
10. 2nd Lt. L. A. L.	19	Pilot.	9	340	100	35	142/60	72 80 90	Feeling strain. Getting jumpy and irritable. No energy. Sleeping badly.

11. Capt.H.S.M.S., M.C.	22	Flight Commander.	12	260	110	70	130/70	72 85 88	Feels perfectly fit.
12. Lt. A. F. McG., M.C.	19	Pilot.	11	315	100	33	118/78	88 100 110	Tired seven weeks. Restless and unsociable. Dyspnoea above 6,000 feet.
13. Capt. J. P.	22	Flight Commander.	10½	400	102	34	115/85	70 82 93	Jumpy one month. Cannot bear to watch machines landing.
14. Lt. G. B. P.	19	Observer.	6	180	150	50	125/72	84 99 106	Fed up with observing.
15. Lt. J. J. Q., M.C.	21	Pilot.	6	180	90	32	116/80	70 87 101	No energy. Restless. Sleeping badly. Talking in sleep.
16. Lt. L. V. S.	19	Observer.	6	135	180	40	140/100	81 88 100	Not up to the mark lately. Sleeping poorly. Tired after long flights.
17. Lt. D. W. S.	19	Pilot.	9½	380	115	38	132/72	—	Feels fit—could do another two months.
18. Capt. R. D. S., M.C.	26	Flight Commander. Teetotaller.	10	350	110	35	—	72 80 93	Tired out. Sleeping badly lately. Bronchitis.
19. Lt. D. S. T.	24	Pilot.	9	400	90	48	120/75	74 80 94	Latterly tired after flying. Otherwise feels fit.
20. Lt. J. T.	24	Observer.	4	200	100	48	—	63 71 76	Badly crashed two weeks ago. Nervous ever since.

TABLE I. PILOTS AND OBSERVERS TRANSFERRED TO HOME ESTABLISHMENT FOR A REST

(*c*) ARTILLERY PILOT AND OBSERVERS (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Remarks.*	*Months since posted Overseas.*	*No. of Hours War Flying.*	*Expiratory Force in mm. Hg.*	*Time in Seconds of Sustaining 40 mm. Hg by Blowing.*	*Blood Pressure.*	Pulse. *Sitting. Standing. Exercise.*	*Remarks.*
21. Lt. J. H. U., M.C.	20	Observer. Rejected by C.B. as Observer.	11	215	118	33	110/70	70 83 99	Feels very fit. Began to get tired five months ago, but was restored by leave.
22. Lt. S G. W.	27	Observer.	5	146	85	55	—	81 94 115	Sick in air lately. Sleeping badly. Tired and run down.
23. Capt. F. F., M.C.	22	Flight Commander 400 hours instructing.	13	600	115	45	120/78	65 82 84	Everything is an effort. Irritable and unsociable. Hates his work.
24. Lt. H. S. W.	24	Pilot.	3	150	70	35	—	79 85 91	Heavy work lately—low strafing. Feels rattled.
				AVERAGE	119·3	43·5	125/80	71 84 94	

(*d*) NIGHT-FLYING PILOTS AND OBSERVERS

1. Lt. T. H. D. S.	19	Observer.	5	100	110	45	110/60	68 85 96	Many forced landings lately. Sleeping badly. Tired. Very keen to learn to fly.
2. Lt. M. S.	26	Observer.	6	104	120	65	120/90	58 67 88	Headaches lately. Keen to learn to fly.

3. Capt. A. L. C.	27	Flight Commander. 200 hours H.D.	2	60	125	55	148/100	108 123 147	No leave for 18 months. 'Flu' three weeks ago. Now D.A.H.
4. Lt. L. D. P.	19	Observer.	3	70	72	47	120/70	95 110 115	Numerous forced landings lately Pilot killed. Restless and sleepless. Very jumpy.
5. Capt. P. G. S.	23	Flight Commander.	3	400 obs. (1916) 120	70	37	136/95	52 60 72	Irritable and restless. Never used to be like this.
6. Lt. E. R. H. B.	26	Pilot. a/Flight Commander.	4	118	120	50	125/95	65 82 101	Eight forced landings in 14 days. Very tired. Returned one month later. Did 50 hours more, then to H.E. with exp. force 90 and fatigue test 42.
7. Lt. A. H. G. D.	22	Pilot.	6	150	90	50	120/65	66 76 79	Feels as fit as ever.
8. Capt. W. H. T.	26	Flight Commander. 200 hours H.D. 100 hours instructing.	2	425 obs. (1916–17) 170 (1917) 60	70	46	124/90	94 106 118	Worked to death—never had any decent leave. Wants to jump out of machine.
9. Lt. S. C. H.	25	Pilot.	6	100	80	30	112/60	80 88 96	Three forced landings with bombs on board. Lost confidence. Depressed.
10. Capt. H. M. C.	27	Flight Commander. 500 hours H.D.	8	100	80	30	118/70	51 56 63	Very tired and apprehensive.
11. Lt. B. R. S. L.	21	Pilot.	2	190 obs. (1917) 30	70	30	125/75	80 93 105	No leave for a year. Very tired but keen.
12. Lt. A. L. S.	25	Pilot. 50 hours H.D.	7	90	100	48	122/74	68 77 85	Headaches. Stale. Giddy in air lately.
13 Lt. T. T., D.S.C.	19	Observer.	12	200	100	35	130/90	78 100 107	Excitable type. Very tired.

TABLE I. PILOTS AND OBSERVERS TRANSFERRED TO HOME ESTABLISHMENT FOR A REST

(*d*) NIGHT-FLYING PILOTS AND OBSERVERS (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Remarks.*	*Months since posted Over-seas.*	*No. of Hours War Flying.*	*Expira-tory Force in mm. Hg.*	*Time in Seconds of Sustain-ing 40 mm. Hg by Blowing.*	*Blood Pressure.*	PULSE. *Sitting. Standing. Exercise.*	*Remarks.*
14. 2nd Lt. M. M. T.	32	Observer.	5	40	125	52	127/78	73 104 112	Heavy work last three weeks. Getting jumpy. Thinks he is too old.
15. Lt. J. P.	27	Observer.		85	110	50	134/75	95 102 110	Blind in one eye—very good observer. Tired but keen to learn to fly.
16. Capt. P. W. R	22	Flight Commander.	7	100	110	55	122/95	72 81 92	Not sleeping too well. Lost some of his keenness.
17. 2nd Lt. C. A. McC.	19	Pilot. 40 hours H.D.	6	100	130	40	123/90	87 96 114	Shaky last two months and has palpitation on exertion. D.A.H.
18. Lt. E. W. C.	29	Pilot. 40 hours H.D.	6	100	140	43	110/70	80 89 91	Lived in hot climates. Has had malaria, typhoid, and dysentery. Feels the cold. Bronchitis. Otherwise fit.
19. Capt.M.G.F.R.	25	Flight Commander 400 hours H.D.	5	70	100	38	105/55	66 77 83	Forced landings lately. Tired and lost confidence.
20. Lt. W. R.	26	Observer.	6	100	135	55	112/75	68 89 109	Feeling stale but anxious to learn to fly.
21. Lt. W. F.	24	Observer.	7	100	120	59	135/75	84 97 106	A little run down but nothing to speak of.
				AVERAGE	103·6	45·7	127/78	76 89 100	

As it is reasonable to assume that the respiratory centre is likely to be more affected in those who fly high rather than those who fly low, sub-classifications have been made into (*a*) Bristol Fighter and D.H. pilots, (*b*) Scout pilots, (*c*) Artillery pilots, (*d*) Night pilots. None of the individuals had used oxygen in the air.

TABLE II

Subject.	*No of cases examined.*	*Expiratory force in mm. Hg.*	*Seconds of sustaining 40 mm. Hg.*	*Blood pressure in mm. Hg.*	*Sitting pulse.*	*Standing pulse.*	*Exercise pulse.*
I. All pilots and observers on transfer to H.E for a rest.	91	116·6	46·7	123/78	72	85	95
(*a*) Bristol Fighter and D.H.	20	130·5	49·7	121/74	71	84	93
(*b*) Scouts.	26	108·5	47·2	121/79	70	83	90
(*c*) Artillery.	24	119·3	43·5	125/80	71	84	94
(*d*) Night flying.	21	103·6	45·7	127/78	76	89	100
II. Fit instructors.	22	112	52	—	—	—	—
III. Pilots returned for rest.	17	95	40	—	—	—	—
IV. Pilots taken off flying through stress.	27	74	25	—	—	—	—

It will be seen that the Bristol Fighter and D. H. pilots are the best in the series in both the manometer tests, while scouts are better than the artillery series in the ' fatigue ' test, and not so good in the expiratory force test.

Compared with the results obtained by Flack (*vide* No. 7 of this volume, Table XIII, p. 119), as the table given above shows (Table II), the home establishment series is only slightly below the standard of fit instructors and home defence pilots on the fatigue test, and as good if not better on the expiratory force test. They are immeasurably superior to the series of pilots taken off flying through stress, and markedly superior to pilots ' returned for rest '. From this last comparison we are perhaps justified in hoping that flying officers are now withdrawn from the line before their powers of recuperation have undergone any permanent diminution.

The above results do not warrant the assumption that high flying *per se*, at any rate at the stage at which pilots and observers are nowadays withdrawn from the line, produces an altered tone of the respiratory centre of such a character as to be demonstrable by the manometer tests. It should, however, be remembered that the individuals included in Table I are to a certain extent representatives of the fittest to survive. In this connexion it is not proposed to push the comparison between Bristol Fighter pilots (average 16,000 feet) and night-bombers (average 3,000 feet). It is the general opinion of most wing medical officers that the pilot who has been employed on night work compares unfavourably as regards

TABLE III. CONCUSSION CASES

Serial Number, Rank, and Initials.	*Age.*	*Remarks*	*Hours flown on Active Service*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	*Blood Pressure in mm Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Disposal.*	*Remarks*
1. Lt. H. V. B.	19	Bristol Fighter Pilot.	22	65	19	100/64	83 95 112	England.	Brought down in No Man's Land. Unconscious ½ hour. Much shaken.
2. Capt. P. B., D S C.	22	Handley Page Observer.	120	70	15	115/70	58 68 72	England.	Never able to fly above 10,000 feet. Week ago shot down in sea. Dazed. Pilot drowned. Shaken.
3. 2nd Lt. J. Y. B.	27	D.H. 4. Observer.	40	130	32	138/84	85 88 102	England.	Two crashes last week. Knocked out for few seconds. Hospital since. Very shaky.
4. Lt. R. B. D.	19	Scout Pilot.	20	120	52	125/72	71 87 96	Sick leave.	Crashed taking off. Dazed—not unconscious. Now lost confidence. ? Temperamentally unfit. Did not return.
5. 2nd Lt. F. G.	21	Artillery Pilot.	45	100	28	125/80	90 101 101	England.	Crashed—unconscious five minutes. Headache and lassitude ever since.
6. Major G. P. H.	26	Scout Pilot.	60	140	40	120/75	74 80 87	England.	Crashed one month ago—unconscious five minutes. Headache ever since. Giddy in air and on ground. Insomnia.
7. 2nd Lt. F. A. W. M.	21	D.H. 4. Pilot.	75	75	33	128/68	71 78 83	England.	Machine collided with his on aerodrome. Unconscious ten minutes. Headache and syncopal attacks ever since.

8. 2nd Lt. C. M.	21	Scout Pilot.	140	115	39	120/75	58 68 66	Sick leave.	Two days ago shot down. Unconscious ten minutes. Headache and lassitude. Rapid improvement. Since killed in collision over lines.
9. Lt. H. H. P.	26	Artillery Pilot.	110	120	42	145/115	72 83 92	Sick leave.	Machine caught fire—crashed. Remembers nothing about it. Headache since. Shaken. Returned and did good work for seven weeks.
10. Lt. J. E. S.	19	Artillery Pilot	45	80	55	140/88	99 113 125	Sick leave.	Crashed 12 days ago. Unconscious ½ hour. Headache and loss of energy. Improved. Doing very good work since return.
11. Lt. S. T. T.	18	Scout Pilot.	2	90	36	125/90	76 87 95	England.	Crashed—unconscious three hours. Now feels fit. To have further instruction in flying. Loss of confidence.
12. Lt. L. M. T.	24	Bristol Fighter Observer.	150	130	45	142/110	93 99 106	Sick leave.	Crashed two weeks ago—unconscious 90 minutes. Now feels fit Did well on return.
13. Capt. H. A. W.	21	Scout Flight Commander.	80	110	50	130/90		Duty.	Crashed six days ago—unconscious 15 minutes. Confused 12 hours. Now full out. Since brought down several E.A. Awarded D.F.C. and D.S.O.
14. Lt. C. F. P. H.	19	Scout Pilot.	2	80	25	145/100	96 101 113	England.	Collided in air with Flight Commander, who was killed. Unconscious five minutes. Insomnia and headaches since.
15. Lt. C. H. C.	23	Scout Pilot.	65	160	45	120/70	62 78 88	Sick leave.	Crashed week ago. Unconscious 2½ hours. Headache two days Now feels fit Did well on return.
16. Lt. T. L. B.	20	Artillery Pilot.	130	100	40	135/94	67 76 83	Sick leave.	Shot down—crashed—shelled—stunned ten minutes. Headache since. Did very good work on return. Awarded D.F.C.

TABLE III. CONCUSSION CASES (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Remarks.*	*Hours flown on Active Service.*	*Expiratory Force in mm. Hg.*	*Seconds for sustaining 40 mm. Hg.*	*Blood Pressure in mm. Hg.*	PULSE. *Sitting Standing. Exercise.*	*Disposal.*	*Remarks.*
17. Lt. F. A. S.	20	Artillery Observer.	100	100	50	135/90	75 93 99	Sick leave.	Observer on previous case. Dazed not unconscious. Good work on return. Awarded D.F.C.
18. Lt. D. J. M.	19	Artillery Pilot.	70	65	25	118/78	84 91 114	England.	Crashed three weeks ago—unconscious 45 minutes. Hospital since. Headache. Vertigo. ? Fractured Base.
19. 2nd Lt. C. W. H.	19	Scout Pilot.	200	62	25	115/74	63 79 92	England.	Hit tree — crashed — unconscious few seconds. Now feels fit but lazy.
20. Lt. R. H. R.	25	Scout Pilot.	20	130	40	130/80	60 66 72	Sick leave.	Crashed ten days ago—hit head—dazed. Three days later flew again and found he was useless. Very good work on return.
21. Lt. R. D. D.	20	Scout Pilot.	20	100	30	125/90	76 108 108	England.	Crashed three weeks ago—unconscious ten minutes. Hospital one week. Since lost all confidence in the air.
22. 2nd Lt. F. A. P.	26	Scout Pilot. 2½ years in infantry.	22	80	25	120/65	89 122 155	England.	Two crashes in succession. Last one due to losing his head. Dazed. Headache. Very jumpy.
23. Lt. E. G. McM.	30	Scout Pilot.	25	90	65	115/70	67 93 91	Sick leave.	Crashed ten days ago—unconscious one minute. No confidence next time up. Now fit and keen No headache. Did not return.
24. Lt. K. G. P. H.	19	Scout Pilot.	170	190	60	138/70	—	Duty—to H.E.	Crashed 14 days ago—unconscious three days. Now feels absolutely fit.
			AVERAGE	104·0	38·1	127/81	77 88 99		

fitness with the pilot who flies by day. This result is occasioned partly by the fact that casualties being relatively small, there is more chance of the 'unfit' surviving as opposed to the 'fit'; but mainly by the unnatural mode of life and consequent irregularity of meals, lack of exercise, and in the hot months more especially lack of sleep. This point will be referred to later.

In view of the hypothesis that the 'fatigue test' is an indication of the tone of the respiratory centre, and assuming that the tone of both the higher and medullary centres is likely to be affected by cerebral concussion, 24 cases have been collected in Table III of pilots who have recently been concussed as the result of a crash.

It will be seen that the results are considerably lower than those obtained in the series of tired pilots. Further analysis of the concussion series, however, reveals the interesting fact that if they are divided into two categories, viz. (*a*) those who were evacuated to England, and (*b*) those who were considered to be so little affected as to be likely to be fit for active service after three weeks' sick leave, the latter reach a high level of excellence on the manometer tests, while the former fail.

Of the sick leave series only two failed; both reported sick in England, and were found permanently unfit for flying to the great disgust of one of them (Case 23). The temperamental fitness of the other (Case 4) was, however, open to question. All the ten who returned did excellent work. Case No. 13, in spite of having been unconscious for 15 minutes, was so little affected that he was flying again ten days later, and during the past three months has destroyed several E. A., and been awarded the D.F.C. and D.S.O.

The results set out in Table IV are the more noteworthy in that at the time these officers were in hospital the writer had not seen Flack's paper, nor had he learnt the value of the manometer tests. That is to say, the results of these tests carried little if any weight in arriving at a prognosis.

TABLE IV. CONCUSSION SERIES

Subject.	*No. of cases examined.*	*Expiratory force in mm. Hg.*	*Seconds of sustaining 40 mm. Hg.*	*Blood pressure in mm. Hg.*	*Sitting pulse.*	*Standing pulse.*	*Exercise pulse.*
All concussion cases	24	104·0	38·1	127/81	77	88	99
(*a*) Granted sick leave or returned to duty.	12	120·4	48·6	131/85	74	85	93
(*b*) Evacuated sick to England.	13	87·6	27·6	123/76	79	91	103

Pulse Rate.

Reference to the following table (No. V) shows that the home establishment series is characterized on the whole by a quiet pulse, the rate being considerably faster in night-fliers than day-fliers. In the concussion series the rates are somewhat higher, due entirely to the faster rate in those cases that had to be evacuated sick to England. For the sake of comparison, included in the table is

TABLE V. PULSE RATE AND BLOOD PRESSURE

	1	2	3	4	5	6	7	8	9	10	11
Subject.	*No. of Cases examined.*	*Sitting Pulse.*	*Standing Pulse.*	*Exercise Pulse.*	*Blood Pressure in mm. Hg.*	*Percentage Number of abnormal Pressures.*	*Percentage Number of Systolic Pressures above Normal.*	*Percentage Number of Diastolic Pressures above Normal.*	*Percentage Number of Pulse Pressures above 40 mm. Hg.*	*Percentage Number of Systolic Pressures below Normal.*	*Percentage Number of Diastolic Pressures below Normal.*
I. All pilots and observers to Home Establishment.	91	72	85	95	123/78	68·2					
(*a*) Bristol Fighter and D.H.	20	71	84	93	121/74	65·0	35·0	20·0	30·0	15·0	10·0
(*b*) Scouts.	26	70	83	90	121/79	69·2	38·5	30·7	23·0	19·0	19·0
(*c*) Artillery.	24	71	84	94	125/80	60·0	4·00	25·0	30·0	5·0	5·0
(*d*) Night-flying.	21	76	89	100	127/78	90·5	28·5	38·0	57·0	4·7	19·0
II. All concussion cases.	24	77	88	99	127/81	70·8	45·8	37·5	33·3	4·0	12·5
(*a*) Granted sick leave and returned to duty.	12	74	85	93	131/85	83·3	66·6	50·0	41·6	0	0
(*b*) Evacuated sick to England.	12	79	91	103	123/76	58·3	25·0	25·0	25·0	8·0	25·0
III Permanently unfit for flying.	29	87	100	110	130/82	85·7	50·0	43·0	50·0	0	10·7

a summary of the results obtained in a series of officers found permanently unfit for flying for various reasons ; these, it will be seen, have on the average rapid pulse rates.

Blood Pressure.

This has been analysed as follows, but it must be admitted that the results of blood-pressure estimations are too variable to allow of any very definite conclusions being drawn.

The blood-pressure results have been collated as follows :

a. The average systolic and diastolic-pressures are noted.
b. The percentage number of abnormal readings is noted.
c. The percentage number of systolic-pressures above and below normal, of diastolic-pressures above and below normal, and of pulse-pressures above normal are noted.

The normal limits have been taken as :

i. Systolic—110 to 125 mm. Hg.
ii. Diastolic—70 to 85 mm. Hg.
iii. Pulse pressure—45 mm. Hg or lower.

It will be observed that column 6 is not equal to columns 7 to 11 added together, but considerably less. This is because a blood-pressure reading may be abnormal in more than one direction. For example, a reading such as 170/110 mm. Hg is abnormal in three directions, the systolic, diastolic, and pulse-pressures being all above normal.

The chief points to which attention is directed are first the high average of the systolic-pressures in all groups, and secondly the large percentage of abnormal pressures. The abnormality tends considerably towards high readings than towards low, and is most pronounced in concussion cases granted sick leave and the permanently unfit. These high readings have in no case been associated with arterial or renal changes, and are presumably to be looked upon as an indication of the strain of active service. It is only in high-altitude pilots, night-fliers, and the bad concussion cases that we find an average diastolic-pressure below 80 mm. Hg and any considerable percentage of cases with subnormal blood-pressures. In the night-flying group this is associated with a somewhat rapid pulse and large pulse-pressure, and is probably due to that general lack of condition to which reference has already been made (*vide* Table I, Cases (d) 1, 7, 9, 12). In the high-flying pilots (*vide* Table I, Cases (a) 1, 2, 10, (b) 6, 11, 17, 25, 26) it is more common to find a pulse below rather than above the normal and a normal pulse-pressure, and it is rather tempting to suppose that the condition is a result of repeated exposures to lowered oxygen pressures.

In order to determine the value of the examination employed, a series of officers found in the field permanently unfit for flying has been collected. All these officers had given practical proof of incapacity in the air in some way or another. No attempt is made to analyse the series, which is somewhat heterogeneous in character, including as it does such different disabilities as sickness in the air or shortness of breath on the ground.

TABLE VI. PERMANENTLY UNFIT FOR FLYING

Serial Number, Rank, and Initials.	*Age.*	*Hours flown on Active Service.*	*Remarks.*	*Expiratory Force in mm. Hg*	*Seconds for Sustaining 40 mm. Hg.*	PULSE. *Sitting. Standing. Exercise*	*Blood Pressure in mm. Hg.*	*Remarks*
1. 2/Lt. P. W. A.	20	3	Passed by C.B. as observer only.	50	16	77 89 94	130/70	Undersized; B ii. for ground work. Fainted twice in England at 9,000 feet and once in France.
2. 2/Lt. O. H. B.	19	8	Passed by C.B. as observer only. Congenital Torticollis.	88	3	79 101 112	120/70	Attacks of blood rushing to head and everything goes black. Insomnia 14 days. Category B i.
3. 2/Lt. A.W.M.B.	19	10	When training had to change from scouts to D.H. 4 as stunting made him giddy. Pilot.	50	29	74 111 133	138/94	Very poor physique. Bad peripheral circulation. Giddy in the air. Cannot land properly. Category ? A after training.
4. Lt. T. R. D.	26	8	Always sick at 8,000 feet in England. Pilot.	80	20	68 75 81	125/80	Dizzy and fainted 10,000 feet. Crashed. Test flight fainted 12,000 feet. Pain in ears. Invagination both drums on landing. No permanent Eustachian obstruction. Category A.
5. Lt. H. D.	20	16	Pilot. Not passed by C.B.	78	15	126 129 150	140/100	Cannot face the lines. Twitching of muscles of limbs and face. Profound apprehension. Diffuse sweating. Thyroid not enlarged. Evacuated sick.
6. Lt. C. I. F.	19	29	Sick and giddy when training. Never a stunt pilot.	150	50	78 84 90	112/90	Giddy in the air and increasing dislike of flying of any kind. Ears normal. Category A.
7. 2/Lt. L. L. G.	23	2	Stopped flying scouts in England because he was no good and got giddy when stunting.	—	—	74 89 95	135/78	Views prospect of flying with dread. Cannot sleep or eat. Category A.

8. Sgt. G.	22	30	No shortness of breath. C_2H_5OH. Observer.	100	15	130 146 146	154/85	Bad type. Pilots will not fly with him. No distress on exertion. Alcoholic.
9. 2/Lt. W. R. G.	19	125	Passed by C.B. fit as observer only. D.N.S. resected 1916. Vomited in air in England.	80	24	64 76 79	135/85	Increasing giddiness and nausea in air. Auto-inflation normal. No determination.
10. 2/Lt. P. H.	33	85	Passed by C.B. as observer only. Never been strong.	—	—	67 78 86	130/75	Mitral Regurgitation. Neurasthenia. Category B iii. Shaky and hysterical.
11. Lt. H. V. H.	23	90	No medical examination. Observer.	65	18	120 124 135	168/88	Giddy in air, faints on the ground. Tremor + +. Evacuated sick to England.
12. 2/Lt. E. A. K.	20	15	Mother neurasthenic. Neurasthenia 1916. Classified D 3 for infantry. Passed by C.B. Pilot.	—	—	98 111 113	125/80	When flying comes over all of a tremble. Cannot keep still. Tremor + +.
13. Lt. C. F. K.	25	0	P.H. Rheumatism. Gets hot and sweats all over when flying and has praecordial pain. Pilot.	50	38	101 124 128	125/85	Recently had P.U.O. Fainted twice in convalescence. Not confident about flying. Typical case of the effort syndrome.
14. 2/Lt. R. A. L	20	5	Not passed by C.B. Had to stop playing games at 18. Pilot.	—	—	93 111 118	124/80	Dizzy in air. Cannot spin. Sick if he stunts. No confidence and wind up. Very glad to give up flying. Category A.
15. 2/Lt. G. T. H. L.	23	25	18 months infantry France. No medical examination. Observer.	—	—	87 92 100	122/72	Bad sailor. Sick every time he has flown. Applied to rejoin his regiment. Category A.
16. Lt. R. N. T. L.	22	54	Always been nervous. Bad sailor. Cannot dance. Not passed by C.B. Observer.	70	22	80 94 102	128/75	Giddy in the air; not sick. No determination. Category A.
17. Lt. W. T. M.	28	15	Not passed by C.B. Pilot. Breakdown in 1914. Never fit since and realizes his limitations.	90	39	105 113 127	120/65	Finds flying exhausting. Effort syndrome. Dyspnoea on exertion. B ii.

TABLE VI. PERMANENTLY UNFIT FOR FLYING (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Hours flown on Active Service.*	*Remarks.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Blood Pressure in mm. Hg.*	*Remarks.*
18. 2/Lt. J. L. P.	25	2	No medical examination. Pilot.	—	—	98 113 120	125/96	Always hated flying and said so in England. Cannot look out side of machine, as it makes him giddy. Loses his head landing. Insomnia. Category A.
19. 2/Lt. A. R.	19	8	Passed by C.B. as observer only. Height 5 feet 1 inch. Weight 9 stone.	72	30	93 111 117	125/90	Sick every time he flies, and not tall enough to fight his gun. Category B ii.
20. 2/Lt. T.	18	2	Passed by C.B. Complained in England of giddiness, praecordial pain and dyspnoea on exertion. Pilot.	—	—	70 75 95	135/65	Pants at 6,000 feet, and vomits on landing after stunting. Very distressed after a sharp walk. Fainted at 15,000 feet on bag. Immature, may develop. Category B ii.
21. 2/Lt. C. E. T.	28	20	Passed by C.B. as observer only.	70	21	80 88 106	140/90	Never fit since he began flying. Dyspnoea at 10,000 feet, syncope at 17,000 feet. On Flack's bag fainted at 17,000 feet. Wind up.
22. 2/Lt. J. L. T.	18	20	Passed by C.B. as observer only. Always felt faint and sick in the air in England.	80	35	101 102 107	130/105	15,000 feet using O_2 intermittently—thinks he fainted, and can remember nothing till he landed. Immature, may develop later.
23. Lt. J. H. U.	19	20	Not passed by C.B. All right flying in England. Pilot.	60	25	100 107 123	120/70	Increasing fear of crossing the lines. Short of breath on ground lately. A.B. $\frac{1}{2}$ in. ext. L.N.L. Probably effort syndrome.
24. Lt. C. A. V.	27	30	Rh. Fever 1917. 3 times rejected for infantry. Very bad sailor. Sick in air in England. Pilot.	15	35	87 97 107	134/90	Sick flying. Dyspnoea in air and on ground. Wretched physique. Left Eustachian obstruction. Old T.B. Rt. apex. B ii.
25. 2/Lt. W. W.	24	40	Bad sailor. Sick in air in England. Pilot.	—	—	78 100 120		Sick after one vertical bank. Does not know what would happen if he got into scrap. Category A.

26. 2/Lt. G. W.	25	12	Passed by C.B. as observer only. Bad sailor, cannot waltz.	—	—	72 100 106	125/65	Sick every time he flies. Septic tonsils. Evacuated to England.
27. Lt. G. B. W.	28	30	Passed by C.B. as observer only. Bad sailor. Sick in air at home.	110	30	73 90 95	135/95	Often sick in the air and always so on rough days. Ears normal.
28. Lt. R. N. W.	19	10	Passed by C.B. Not allowed to play games by Doctor's advice. 8,000 feet in England, felt faint and had praecordial pain. Pilot.	40	20	79 97 101	123/90	Nearly fainted at 5,000 feet. Fainted twice on ground recently. Cardiac arrhythmia. Debility. May develop after training.
29. Lt. E. W. W.	19	15	Sick once in England. Pilot.	70	45	85 92 98	125/90	Sick every time he does a vertical bank. Last time fell over on joy stick, spun from 4,000 to 500 feet and was pulled out by observer. Rt. Eustachian obstruction. Taken up on test flight and vomited all over machine. Category A.
			Average			87 100 210	130/82	

Of the 16 pilots, 15, i. e. 94 per cent., had either not been passed by the Commissions Board, or gave a flying history while in England suggesting that their retention as flying officers was at any rate open to question. The remaining pilot, who had been passed fit, was a confirmed neurasthenic with a bad family history, and had already been classified D iii for the infantry. Of the 13 observers, nine only had been before the Commissions Board, *and all of these had been passed 'fit as observer only'*.

Taking the series as a whole it will be seen that the manometer results are bad, the average systolic pressure is well above normal (130 mm. Hg), while the percentage number of high systolic, diastolic, and pulse-pressures is distinctly high. At the same time the pulse rates are greatly in excess of those obtaining in any other series. Compared with the tired Bristol Fighter group, the sitting pulse shows an increase of 22 per cent., the standing of 19 per cent., and the exercise of 18 per cent.

This series may therefore justifiably be looked upon as confirmatory evidence of the value of the special examination employed.

The temperamentally unfit have been divided up into three groups :

(*a*) Those reported as temperamentally unfit by executive officers, an opinion for which no medical confirmation is available.
(*b*) Those in which a diagnosis of temperamental unfitness has been originated or confirmed by medical evidence.
(*c*) Psychopaths.

(*a*) This class is of scientific interest only in so far as its members *pass all the tests* (*vide* Table VII).

Three out of the eight cases returned to squadrons and completely justified their retention (*vide* especially Case 8).

(*b*) It is with this class that we are more particularly concerned. As a class it is characterized by the presence of physical signs. Nearly all the cases appeared to be in a state of trepidation and apprehension, and loss of emotional control was a prominent feature. Unlike the next series, the incapacity was attributable in about half the cases to some particular incident, such as a crash or a narrow escape. The general physique was distinctly below the average, and the ratio of pilots to observers was as 14 to 3. *Of the six observers all had been passed by the Commissions Board 'fit as observer only'*.

It will be seen that on the expiratory force test the series works out at 87 mm. Hg, i. e. well within the standard, but considerably below the figures reached by tired pilots and observers. On the fatigue test, however, the figure (28·8) is not far above that given by a series of pilots taken off flying through stress (*vide infra* and Report No. 7, Table XII, pp. 116–18).

(*c*) *Psychopaths.* The writer is not convinced of the soundness of this term, but proposes to retain it for want of a better one. Individuals belonging to this class usually, but not always, have a neuropathic family or past history. As a general rule they have never been confident in the air from the outset, and at the time

they reach hospital have developed a pronounced anxiety neurosis, the motif of which is flying and not fighting. *All of the cases were pilots.* A history of a crash or a particularly unpleasant experience is rare, and shock is not apparently an aetiological factor. One and all look worried and depressed, taciturn, and disinclined to 'open out', while the majority appear unable to state with any precision why it is they do not like the air. Repression is undoubtedly present, and physical signs are conspicuous by their absence. The disability, in fact, is mental and remains mental, and their decision to give up flying has often struck the writer as being somewhat cold-blooded.

Judged by manometer results, the psychopath retains a high degree of physical fitness, the pulse rate is barely above the normal, the average systolic and diastolic pressure is not high, while out of nine cases there are only two (22·2 per cent.) with supernormal systolic, and only one (11·1 per cent.) with supernormal diastolic readings (*vide* Table X).

Pulse Rate and Blood Pressure.

In groups (*a*) and (*c*) the pulse is not rapid, in fact the former gives the slowest rate of any series. The number of cases examined is not, however, sufficient to allow of any definite conclusions being drawn either as regards pulse rate or blood pressure.

In group (*b*), on the other hand, the pulse rate is on the high side, the average systolic pressure is 130 mm. Hg, while half the cases had systolic pressures above 125 mm. Hg.

Taken in conjunction with the manometer tests, these results show that the temperamentally unfit is unstable physically. The most natural conclusion is that certain individuals are so constituted that they react to strain by alteration of their physical state, or in other words, that by reason of their temperament they will necessarily become physically unfit when exposed to strain. This lack of resistance appears to be a characteristic of the general 'make up' of the individual, and it must be supposed that the lack of inhibition in the higher nervous centres paves the way for a spread of nervous impulses; this in turn gives rise to a variety of physical phenomena, the main feature of which is overaction. Thus we frequently see in the somatic system overaction of various neuro-muscular groups, more especially of the speech mechanism, while in the autonomic system it is displayed by overaction of the heart (rapid and disordered action), of the skin (sweating), of the gastric mucosa (hyperacidity), and possibly of the adrenal glands (raised blood pressure), while it is also not impossible that the loss of weight which frequently occurs in these cases is due to a general increase of metabolism secondary to changes in the autonomic system.

The practical proof that an individual of this type is unfitted for service flying is supplied by his failure to withstand mental strain of such a degree as to affect the more stable individual only temporarily or not at all, while confirmation of his instability is provided by physical as well as mental examination. Temperamental and physical unfitness are accordingly not separate but closely allied things, and the patient's frequent statement, 'I cannot go on,' accurately sums up the situation.

TABLE VII. TEMPERAMENTALLY UNFIT (*a*)

Serial Number, Rank, and Initials.	*Age.*	*Hours flown on Active Service.*	*Type of Machine.*	*Previous History.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Blood Pressure in mm. Hg.*	*Remarks.*
1. Lt. H. B. P.	19	93	Scout.	Passed by C.B. Pilot. 210 hours as instructor.	85	45	70 88 91	120/82	Slight crash two days ago. Dazed; sleeping badly, ten days. No loss of confidence in air. Quite fit on discharge.
2. Lt. A. C.	19	30	Bristol Fighter.	Pilot.	85	55	73 86 94	140/80	Complains of dyspepsia. Flatulence and dizziness at any great altitude. Has had to return from patrols. No physical signs.
3. Lt. P. S. B.	20	60	Bomber.	Pilot.	90	42	70 76 86	110/70	Ordinary symptoms of oxygen want during and after long shows at 18–20,000 feet.
4. 2nd Lt. D. S.	22	14	Bomber.	Observer.	170	60	64 74 76	136/85	Headache after high flying.
5. 2nd Lt. A. E. B.	22	42	Artillery.	Pilot.	120	35	65 75 86	132/70	Shot down one month ago. Since then feels sick in the air. Returned to another sqdn. and is doing well.
6. 2nd Lt. C. R. A.	23	20	Artillery.	Joined as Pilot against his will. Never been at all keen on flying.	—	—	71 82 88	105/65	Feels 'unwell' in the air.

7. Lt. J. E. H. C.	19	42	Bomber.	Previously done a little artillery work and liked it. Observer.	90	42	71 86 98	112/85	Cannot look over the side at 10,000 feet, gets horrible sensation of height; seems isolated from the earth and almost wants to jump out. Returned to Artillery Squadron and since qualified as F.O.O.
8. Lt. J. L. R.	19	35	Scout.	Reported as T.U. by Sqdn. Cmdr. in 1917; not agreed with on medical grounds, largely on account of age. Retained in R.F.C. and was posted to a sqdn. as gunnery officer. Felt he wanted to 'make good' in the air. Passed fit.	100	35	67 74 84	145/105	Brought down 9 E.A. in about three weeks and was then killed in aerial combat. Posthumous award of D.F.C.
				AVERAGE	106	45	69 80 88	125/81	

Three out of the eight cases returned to squadrons and completely justified their retention (*vide* especially case No. 8).

TABLE VIII. TEMPERAMENTALLY UNFIT (*b*)

Serial Number, Rank, and Initials.	*Age.*	*Hours flown on Active Service.*	*Type of Machine.*	*Previous History.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Blood Pressure in mm. Hg.*	*Remarks.*
1. Lt. D. R. B.	19	45	Bomber.	Accepted by C.B. as observer only.	85	25	68 81 84	115/65	Shot down three weeks ago—uninjured. Since then lost all confidence, and could not face lines. Lost weight recently. Bad physique.
2. Lt. H. McE.	25	?120	Artillery.	Pilot.	72	25	87 97 107	145/115	Giddy in the air—no nausea. Lost confidence. Bad type. C.O. reports very adversely.
3. Lt. B. P. T.	22	25	Bomber.	Accepted by C.B. as observer only.	70	40	66 75 86	120/80	?Hit by splinter from A.A. 14 days ago. Giddy in air. Not much interest in flying.
4. 2nd Lt. F. W. F. T.	24	50	Artillery.	Accepted by C.B. as observer only.	80	35	115 127 124	170/110	Has gradually found strain of service flying too much for him. Collapsed in cock-pit when he saw E.A. approach. Now wind up.
5. Lt. C. M. D.	26	17	Scout.	Pilot.	120	20	66 85 95	113/80	Insomnia and nightmares. Will not admit loss of confidence.
6. Lt. S. W. C.	23	65	Scout.	Occasional vomiting in air. Previously examined following ?syncope at 4,000 feet and crash.	80	35	68 85 90	118/65	Feels faint at 12,000 ft. Finds flying a great strain, and thinks he will never make a scout pilot. Much worse since slight crash.
7. Lt. R. D. S.	23	20	Night.	Pilot.	110	30	95 99 117	115/85	Has never liked flying and has no wish to continue. Loses himself in the air and does not know position of machine.

8. Lt. R. P.	24	60	Artillery.	Never played games. Pilot.	50	20	65 70 75	145/100	Never felt confidence since coming to France and has to fly by his instruments.
9. Lt. S. H. E.	19	30	Artillery.	Passed by C.B. Crashed three times as pupil.	110	25	65 73 80	140/100	Completely lost confidence since being chased by two E.A. Sleeps well.
10. Lt. B. H.	21	5	Scout.	Took nearly a year learning to fly.	80	27	84 91 100	143/95	Cannot face the air. Hates sight of machine. Lachrymose; not sleeping well.
11. 2nd Lt. C. P.	18	12	Bomber.	Passed by C.B. as observer only.	50	25	78 81 89	115/85	Headache and praecordial pain 12,000 feet. Shot at his own formation. Very tremulous. Immature, may develop.
12. Lt. E. E. B.	22	10	Artillery.	Several crashes in England. Pilot.	—	—	74 76 80	123/80	Feels nervous. Thinks flying a great strain, but is keen to have another shot at it. Feels sorry for his observer. Several crashes due to bad flying.
13. Lt. B. G. B.	24	30	Artillery.	No previous combatant service. Pilot.	85	47	85 100 109	132/75	On two occasions 'things have gone black' in the air. Stammering one week. Lost all confidence.
14. Lt. J. R. B.	22	25	Bomber.	All right flying in England. 120 hours. Pilot.	105	25	90 93 108	115/80	Lost all confidence since seeing a machine land without its undercarriage. Has no wish to continue.
15. Lt. G. A. H.	24	? 120	Artillery.	Previously under observation on posting overseas with tachycardia. High B.P. and general apprehension. Pilot.	110	35	76 94 97	130/90	Felt better after his first flight in Sqdn. Now has little enjoyment in flying.
16. Lt. R. F. C. M.	21	45	Bomber.	V.D.G. 1916. Pilot. 100 hours in England.	40	25	64 81 98	112/60	Shot down week ago—uninjured. Since then great loss of confidence in air, and disinclination to cross lines. Some emphysema.
17. Lt. R. L. P.	19	30	Bomber.	Passed by C.B. Pilot. Bad physique. 120 hours in England.	105	35	72 81 90	148/75	Crashed week ago—no injury. Vivid memories. Insomnia, nightmares, apprehension. Very tremulous.

TABLE VIII. TEMPERAMENTALLY UNFIT (*b*) (*continued*)

Serial Number, Rank, and Initials.	*Age.*	*Hours flown on Active Service.*	*Type of Machine.*	*Previous History.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Blood Pressure in mm. Hg.*	*Remarks.*
18. Lt. S. S. C.	28	12	Bomber.	Passed by C.B. Pilot.	45	15	77 89 104	125/80	Has never liked flying and has no wish to continue, as he cannot face the lines and admits to being terrified.
19. 2nd Lt. C. B. D.	20	45	Night.	Passed by C.B. Pilot 100 hours in England.	55	20	96 103 105	148/100	Crashed month ago—dazed—hospita since. Nightmares. Very tremulous. No headache. Does not think he can stick it.
20. 2nd Lt. H. J. C.	19	160	Bomber.	Passed by C.B. as observer only. ? Rh. Fe. at 7.	45	22	66 75 100	135/90	Two days ago felt giddy after scrap—was using O_2. Felt giddy ever since. Ears normal. Ht. nil except sinus-arrhythmia. Adverse report from C.O.
21. Lt. W. R. T. C.	22	½	Bomber.	Passed by C.B. as observer only. Never been strong enough to take active exercise. History of nervous debility. Sedentary life.	65	25	70 77 86		Congenita Ptosis (Rt.). Very tremulous. Sick in the air. Could not go on under any circumstances whatever. Would like to get back to civilian life.
22. Lt. E. F. S.	28	0	Bomber.	Has lived in South America. Pilot.	140	20	65 79 85	102/78	Pains in back—would prefer to go to warmer climate.
23. 2nd Lt. E. C. H.	21	120	Artillery.	Slight nervous breakdown 1915, driving cars at night. Pilot.	65	28	89 105 108	104/70	Completely lost confidence in the air. Now physically unfit G.S.; requires tonsillectomy.

24. Lt. J. T. B.	20	½	Scout.	Quite fit in England. Pilot.	130	35	77 101 106	116/85	Broke down first flight over lines and could not have faced lines again under any conditions.
25. Lt. A. N. P.	20	? 150	Artillery.	Pilot.	90	30	86 93 99	130/70	Headache after flying. Loss of confidence. C.O. reports—'Has never done a good job of work'.
26. Lt. G. P. L.	19	70	Bomber.	Has done some good work. Pilot.	130	30	93 100 106	125/80	Very erratic in air—several minor crashes. Finds he cannot stand the strain, lachrymose.
27. Lt. W. S. E.	20	70 + 20	Bomber.	Did 70 hours on scouts, and transferred to 2-seater for fear of getting an E.A. on his tail. Pilot.	112	40	98 102 122	143/74	Thinks his vision is bad. V.A. 6/5 R & L. Entirely devoid of offensive spirit.
28. Lt. E. R. S.	27	40	Scout.	Seen active service with infantry and R.F.A. Pilot.	100	40	70 76 80	155/90	Says he enjoys flying, but appears lacking in determination.
				AVERAGE	87	28·8	80 89 98	130/83	

TABLE IX. TEMPERAMENTALLY UNFIT (*c*

(Psychopaths)

Serial Number, Rank, and Initials.	*Age.*	*Type of Machine.*	*Hours flown on Service.*	*Previous History.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	*Blood Pressure in mm. Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Remarks.*
1. Lt. C. M.	25	Scout.	25	Severe insomnia for three weeks at 15.	110	50	110/75	81 88 96	Quite happy in air, but thinks and dreams of flying all night. Profound insomnia. Worried and depressed.
2. Lt. E. N. A. R.	23	Artillery.	160	No war flying for several months. Pilot.	170	45	110/75	67 80 83	Never really happy in air. Increasing loss of confidence. Headache and insomnia. Looks very worried.
3. Lt. J. W. C.	24	Artillery.	15	Pilot.	170	75	115/75	64 74 78	Never happy in the air. Minor crash week ago destroyed all confidence he ever had. No wish to continue.
4. Lt. F. C. C.	23	Artillery.	100	Pilot. V.D.(G.) Aug. 1917.	95	38	138/84	84 94 101	Not fit since V.D.G. Gradually losing confidence. Now cannot face the air. Depressed.
5. Lt. G. W. M.	25	Bomber.	30	15 months with infantry in France. Pilot.	90	43	138/100	88 98 103	Shaky every time he flies. Cannot do a turn. Flying has always worried him. Depressed.

6. Lt. C. J. B. F.	20	Bomber.	70	Pilot.	95	40	120/70	72 89 94	Machine shot about four days ago. Now could not possibly face lines. Sleeps well—no dreams. Feels fit.
7. Lt. J. W. K.	27	Bomber.	15	Rejected by C.B. Passed by Admiralty. Pilot.	110	30	118/85	75 91 100	Flying has always been a great effort and now has no wish to continue. Tried to jump out at 10,000 ft.
8. Lt. W. B. B.	22	Scout.	12	Passed fit in U.S.	85	60	120/70	64 85 99	Persistent headache. Cannot get over seeing bodies of occupants of hostile machine brought down behind our lines.
9. Lt. A. F. M.	25	Scout.	15	Passed fit in U.S.	85	54	120/75	69 86 96	Has never been happy in the air, and now hates it.
				AVERAGE	112	48·2	121/79	75 87 94	

TABLE X. TEMPERAMENTALLY UNFIT

Pulse Rate and Blood Pressure

Subject.	*Number of Cases examined.*	*Sitting Pulse.*	*Standing Pulse.*	*Exercise Pulse.*	*Blood Pressure in mm. Hg.*	*% Number of abnormal Blood Pressures.*	*% Number of Systolic Pressures above Normal.*	*% Number of Diastolic Pressures above Normal.*	*% Number of Pulse Pressures above 45 mm. Hg.*	*% Number of Systolic Pressures below Normal.*	*% Number of Diastolic Pressures below Normal.*
(*a*) By Executive Officers.	8	69	80	88	125/81						
(*b*) By Medical Officers.	28	80	89	98	130/83	67·8	50·0	36·0	39·3	7·1	10·7
(*c*) Psychopath.	9	75	87	94	121/79						

Case 4 (Table IX) is an admirable example of the degree to which the emotions can be reflected in the cardiac-vascular system. He frankly admitted being frightened at the idea of flying over the lines, had a standing pulse of 127, and a blood pressure of 170/110 mm. Hg. Cases 2, 9, 10, 17, 19, 20, and 27 also show the same condition on a smaller scale. Case 19 was posted back to a squadron, but on arrival was unable to take a machine off the ground, to the great relief of his observer.

Discussion of Results.

It has been shown that pilots and observers whose condition, in the opinion of their commanding officers, warrants their transfer to England for what is somewhat euphemistically termed a rest, preserve a comparatively high degree of physical fitness as judged by the tests employed. The chief difference between individuals who have done their work at high as compared with those who have flown at low altitudes lies, not as might perhaps be expected in a poor performance on the manometer test, but in the more frequent occurrence among the former of blood pressures below the normal, with which is usually associated a small and rather slow pulse. Should this finding be confirmed, it not improbably owes its origin to an alteration of tone of the medullary centres brought about by repeated exposures to rarefied atmospheres.

Night-fliers, although having less hours in the air to their credit, are on the whole less fit than day-fliers, a condition attributable to their abnormal mode of life, lack of exercise, sleep, and so on (*vide* Table XI).

Other groups in which the manometer results attain a high level are : (i) individuals who have recently sustained concussion which has affected them so little as to warrant their return to duty either in a few days or after three weeks' sick leave, (ii) psychopaths who do not wish to continue flying, and (iii) a few cases reported on as temperamentally unfit by executive officers in which medical confirmation is lacking.

All these cases are about equally fit, judged by the manometer and pulse rates.

This leaves three groups, to which we will add for the sake of comparison a fourth, viz. Flack's series of ' pilots taken off flying through strain '.

i. Concussion cases evacuated to England.
ii. Permanently unfit for flying on medical grounds.
iii. Temperamentally unfit (excluding psychopaths).
iv. Pilots taken off flying through stress.

It will be seen from Table XII that, judged by the manometer, these groups are about equally unfit and have pulse rates considerably above normal, and the question arises, Have these results an explanation in common ?

As regards the test for expiratory force, it would appear to the writer to depend chiefly on the power and tone of the expiratory and abdominal muscles, together with the ability of the individual

TABLE XI

Subject.	*No. of Cases examined.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	*Sitting Pulse.*	*Standing Pulse.*	*Exercise Pulse.*	*Blood Pressure in mm. Hg.*	*% No. of abnormal Blood Pressures.*	*% No. of Systolic Pressures above Normal.*	*% No. of Diastolic Pressures above Normal.*	*% No. of Pulse Pressures above 45 mm. Hg.*	*% No. of Systolic Pressures below Normal.*	*% No. of Diastolic Pressures below Normal.*
I. Day-fliers due for rest.	70	120·5	47·0	71	84	93	122/78	65·0	37·3	25·4	27·0	13·4	12·0
II. Night-fliers due for rest.	21	103·6	45·7	76	89	100	127/78	90·5	28·5	38·0	57·0	4·7	19·0
III. Concussion cases granted sick leave.	12	120·4	48·6	74	85	93	131/85	83·3	66·6	50·0	41·6	0	0
IV. Psychopaths	9	112·0	48·2	75	87	94	121/79	44·4	22·2	11·1	33·3	0	0
V. Reported temperamentally unfit by executive officers.	8	106·0	45·0	69	80	88	125/81	—	—	—	—	—	—

TABLE XII

Subject.	*No. of Cases examined.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	*Sitting Pulse.*	*Standing Pulse.*	*Exercise Pulse.*	*Blood Pressure in mm. Hg.*	*% No. of abnormal Blood Pressures.*	*% No. of Systolic Pressures above Normal*	*% No. of Diastolic Pressures above Normal.*	*% No. of Pulse Pressures above 45 mm. Hg.*	*% No. of Systolic Pressures below Normal.*	*% No. of Diastolic Pressures below Normal.*
i. Concussion cases evacuated sick.	12	87·6	27·6	79	91	103	123/76	58·3	25·0	25·0	25·0	8·0	25·0
ii. Permanently unfit for flying on medical grounds.	29	76·0	28·0	87	100	110	130/82	85·7	50·0	43·0	50·0	0	10·7
iii. Temperamentally unfit (excluding Psychopaths).	28	87	28·8	80	89	98	130/83	67·8	50·0	36·0	39·3	7·1	10·7
iv. Pilots taken off flying through stress.	27	74	25	—	—		—	—	—	—	—	—	—

to make a properly co-ordinated movement of expiration. It is difficult to assess its value and to gauge its significance, but as an empirical test it is clearly of considerable practical utility. It is interesting to recall that this test is in use in physical training schools in America, and an American pilot, who recently came under observation, recognized it as an old friend, and proceeded to blow the mercury out of the top of the tube (i. e. about 320 mm. Hg) without apparent effort, a performance which rather suggests that there is some ' knack ' about it. The writer, although having a vital capacity of 5,000 c.c., and being above rather than below the average at standing the effects of high altitude, has never succeeded in passing Flack's minimum, i. e. 80 mm. Hg, although he can sustain 40 mm. Hg for nearly 70 seconds.

It is not proposed to enter into a discussion regarding the physiological significance of the ' fatigue ' test, especially as the observations on the behaviour of the pulse during the test, large numbers of which have been made by Captains Corbett and Bazett, have not been collected. Certain points, however, merit attention. Firstly, it appeals to the sporting spirit, and the individual endowed with this is likely to do better than the one who is not. Secondly, it looks much easier than it really is, and the patient may consequently get a false impression of the effort required, and fail to carry out the necessary ' preliminaries '. As a result he does poorly at his first attempt, and makes amends at his second. This discrepancy has often occurred, and is looked on as an unsatisfactory point in the test.[1] Thirdly, determination is undoubtedly an important factor. In other words, the test is in some respects a psychological one.

Returning to the results set forth in Table XII, the question inevitably arises, What is stress, i. e. the condition met with in group iv ? Clearly not the same condition which is found in the tired flying officer on transfer to England for a rest, nor can it be due to the effects of altitude alone, since it occurs in low fliers. An examination of Flack's Table XI, No. 13 of this series, pp. 22–4, reveals the fact that some of the cases (Nos. 7, 16, 21, 24, 25) had done comparatively little flying. Of these, No. 20 only flew once in France, and fainted at 14,000 feet. He was examined by the writer and was found to be suffering from the ' effort syndrome ' (sitting pulse 140, exercise pulse 180) with enlargement of the left ventricle. He had never been able to play games on this account, and was sent home labelled ' permanently unfit '. Further, two cases (Nos. 1 and 2) and perhaps three (No. 7) had been doing long reconnaissances at 20,000 feet, without oxygen. At one time such cases were unfortunately common, but the writer has manometer records of two only (*vide* Table XIII).

It is well known that certain individuals who have done much high flying without oxygen gradually find the altitude at which symptoms

[1] This may perhaps be explained physiologically. During the first attempt owing to the large positive pressure in the thorax the large veins become filled, and this congestion on the right side of the heart may persist for a considerable time. Hence the second attempt is begun with a higher venous pressure and larger inflow into the heart.

TABLE XIII

Serial Number, Rank, and Initials.	*Age.*	*Remarks.*	*Hours flown in France.*	*Expiratory Force in mm. Hg.*	*Seconds for Sustaining 40 mm. Hg.*	*Vital Capacity in c.cs.*	*Blood Pressure in mm. Hg.*	PULSE. *Sitting. Standing. Exercise.*	*Remarks.*
1. Flt. S/Lt. R. P.	22	Pilot, D.H. 4.	200	50	15	4,000	—	—	Long reconnaissance at 18–21,000 ft. without oxygen. Gradual onset of disability to fly high. No loss of confidence.
2. 2/Lt. J. K. C.	20	Pilot, D. H. 4	120	50	19	4,000	—	—	Long reconnaissance at 18–21,000 ft. without oxygen. Feels effects after 3 weeks.
3. 2/Lt. E. S. P.	21	Pilot, D.H. 4. Malaria in Salonica. Still has attacks.	100	60	15	4,000	105/60	69 76 85	Long reconnaissance at 18–21,000 ft. Always uses oxygen when he can get it, as he feels done in without it. Fainted at 14,000 ft. using oxygen, but feeling no benefit. Cannot remember landing. Persistent headache and lassitude. Generally run down.
4. 2/Lt.W. F. N.(D.C.M.)	30	Observer, D.H. 4. Active service chiefly with infantry since 1914. Egypt, Gallipoli, and France. Always been very fit.	154	50	20		135/95	72 81 89	Long reconnaissance at 20–21,000 ft. Oxygen made him sick, so discontinued it, in spite of headache. Last two months has gradually got worse at heights. Now gets periodic breathing above 15,000 ft., rapidly exhausted by slightest movement, can hardly walk on landing and has to lie down, short of breath on ground, never free from headache. No energy. Could sleep for a week. No loss of confidence. Enjoyed the war until recently.
		Average		52·5	17·25	4,000			

of oxygen-want supervene getting progressively lower, a condition which may persist for many months at least. The physiological explanation of this condition is at present undetermined. In all the cases investigated it was associated with evidences of fatigue with disinclination for effort, both mental and physical, and it is therefore not improbable that the condition is primarily cortical in origin.

As regards the four last-mentioned groups, some factor other than altitude must be sought to explain the results, and the most reasonable supposition is that in certain of the cases flying did not cause but merely unmasked a pre-existing condition. It is at least remarkable that if we take a series of pilots who have recently sustained concussion, we find that half of them are fit to return to duty in three or four weeks and pass the manometer tests, while the other half, who are more severely affected, and have to be evacuated to England, fail to pass the tests. These results cannot be explained by a greater degree of concussion in the latter, and they would therefore appear to be due not so much to the actual concussion as to the constitution of the individuals concussed. In other words, it is not concussion of the brain which chiefly matters, but concussion of the mind. In the same way in the temperamentally unfit, and to some extent in those found permanently unfit on medical grounds, the unfitness lies largely in an undue susceptibility to mental shock, to which these individuals react primarily by various mental and psychical phenomena, and secondarily by various alterations in their physical state.

It is here that the importance of determining the pulse-rate and blood-pressure comes in. This investigation has not aimed at studying the mechanical efficiency of the cardio-vascular system or of estimating the response of the heart to exercise and so forth. This has already been done by Starling in the laboratory and by Lewis in the ward. Remembering that emotional disturbances are readily reflected in the circulatory apparatus, it is permissible to look to this apparatus for information in the matter of ' temperament '. The results show that whereas the normal stable individual, when due for a rest after 150 to 500 hours' war-flying, preserves a comparatively slow pulse and a resting blood-pressure within normal limits, the unstable individual with little war-flying to his credit, who has failed to rise to the occasion or has succumbed to some minor accident, has a definitely faster pulse, and a blood-pressure which is frequently abnormally high (e. g. 170/110 mm. Hg).

Lack of resistance to strain appears to be part of the general ' make up ' of the individual, and it must be supposed that the lack of inhibition in the higher nervous centres paves the way for a spread of nervous impulses, which give rise to a variety of physical phenomena, characterized by overaction (tachycardia, tachypnoea, sweating, gastric hyperacidity, arterial hyper-tension, and so on). It is believed that this test has a value altogether apart from flying in general and from high flying in particular ; that this value consists in the assistance which it affords in assessing both the degree of shock sustained by the nervous system and also the susceptibility to shock. Looked at in this way it is a test not so much for fatigue

as for fatiguability, and it is suggested that certain conditions which are at present called ' flying stress ' could more appropriately be designated by the title ' flying distress '.

Conclusions.

1. The majority of pilots and observers transferred to England for a rest preserve a comparatively high degree of physical fitness, day pilots being fitter than night pilots.

2. The flying temperament is of less importance in the field than the fighting temperament.

3. A study of the pulse-rate and blood-pressure together with the manometer tests affords valuable information in assessing temperament and an individual's susceptibility and reaction to mental trauma.

4. A distinction should be made between flying stress and flying distress.

9.—RESPIRATORY TESTS FOR ABILITY TO STAND HIGH ALTITUDES

BY

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Recently a number of airmen (seventy-three) have been examined by respiratory tests, including officers who have proved themselves to be efficient at high altitudes as well as many who have failed to stand this strain. The former class have usually done a large number of hours' flying and have come to hospital because of the resulting exhaustion, whilst the latter have often done very little active-service flying, so that the two classes are not very comparable. None the less, very marked differences are seen, and the results seem worth recording, since those who have proved to be good at high altitudes are found to be much fitter than the others, as judged by these tests, in spite of the fact that they are much more exhausted.

The tests to which they have been subjected are as follows :— their expiratory force has been determined by the U-tube, as well as their power to withstand fatigue when maintaining the mercury at 40 mm. ; their vital capacity, tidal air, and respiratory rate have been measured, and the ventilation per minute (i.e. the amount of air breathed per minute) has been noted. From the results obtained it would appear that these latter measurements are also very valuable in estimating a man's power of enduring oxygen-want.

These methods of examination were adopted as the result of observations on patients during Dreyer altitude tests (see Report 4 of this volume), since it was found that those who reacted to want of oxygen by quickening rather than deepening their respiration were almost invariably bad at standing altitudes,[1] as also were those with a low vital capacity, or very high air consumption when sitting at rest.

A. Methods.

The methods used with the manometer need not be elaborated. It has been employed according to Flack's directions. (See Report 7 of this volume.)

The vital capacity has been determined by using an ordinary Siebe-Gorman 2·5 litre water-meter for measuring gases, any over-blowing of the meter being prevented by the use of a football bladder as a safety-valve. A mouthpiece which can be removed and disinfected is connected to the meter by rubber-tubing in which

[1] The references in the tables to altitude tests refer to cases described in Report 4 of this volume.

a T-tube has been inserted, and to the third limb of the T-tube a football bladder is attached. Even with the bladder inserted in this way the meter may still be overblown if care is not taken; but if the resistance of the tubes between the patient's mouth and the meter is made rather greater than that to the bladder, most of the air passes to the bladder and only the overflow to the meter. With this precaution overblowing of the meter is impossible. At the end of the expiration a light clamp is put on the rubber tube near the mouthpiece, and then the air in the bladder is expelled through the meter. In this way quite accurate results can easily be obtained.

The ventilation per minute has been determined by making the patient breathe through a system of valves, so that the expired air passes through a Siebe-Gorman meter. This creates some resistance to expiration, and to obviate this an ordinary air-cushion

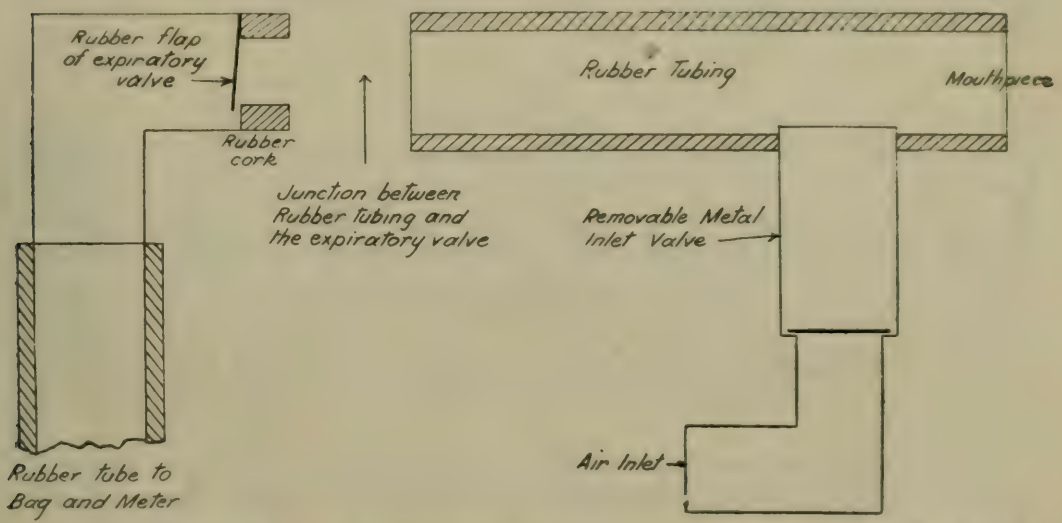


DIAGRAM SHOWING TUBE MOUTHPIECE DISCONNECTED FROM EXPIRATORY VALVE.

is inserted as a bag, making again a T-connexion with the tube between the valves and the meter. The patient is made to wear a nose-clip and to breathe through this system for two minutes, during which time his respirations are carefully counted. At the end of the time the tube is clamped near the mouthpiece, and the air-cushion is emptied through the meter, the total amount of air breathed in two minutes being thus measured. In this way the ventilation per minute is obtained. Some difficulty was experienced in obtaining valves which would be efficient and yet could be easily sterilized. Recently a piece of $\frac{3}{4}$″ bore rubber-tubing four inches in length has been employed as a mouthpiece, as this can easily be sterilized. Its connexion with the inlet and exhaust-valves may be seen in the diagram. The inlet valve used has been the light expiratory valve from a French 'Tizot' gas-mask, the valve being arranged so that it opens only during inspiration instead of expiration; the expiratory valve has been made by using a light flap of rubber sheeting. It is probable that in using these valves some slight leak occurs, especially in quiet breathers, so that the ventilation values obtained may not be absolute, but they serve

sufficiently well to give a good relative test for comparison of different airmen, and compare closely with figures given by Haldane and his co-workers.

The respiratory rate is determined by counting the respirations during the time that the patient is breathing through the meter. If this rate differs widely from a normal respiratory rate, then his rate when breathing naturally at rest is also observed for comparison. Slowing of the respiratory rate as the result of breathing against the slight resistance of the apparatus is fairly often seen in good types, while a quickening of the rate is only found in those who are unable to stand altitude well, or in those who are temporarily ill (e.g. influenzal debility).

The tidal air is determined by dividing the amount of air used per minute by the number of respirations. With the good types the average tidal air found was 523 c.c. and the average respiration rate 14·1, thus affording some confirmation that the methods in use give fairly normal figures.

B. Results.

In Tables I, II, and III may be seen collected the results that have been obtained by these methods. In Table I are included those who have proved themselves to be efficient at high altitudes, though the figures are somewhat affected by the inclusion of many very tired men. Table II contains those who are known to have been bad at high altitudes, while Table III consists of a few whose condition at high altitudes has never been put to the test, together with some border-line cases suffering from mild symptoms of oxygen-want.

It will be noticed that considerable differences exist between these classes, and the results with the different tests may be discussed shortly.

U-Tube Manometer. An average expiratory force of 96 mm. was found in those good at high altitudes, of 81·8 mm. in those who were bad, and 100·8 mm. in those whose behaviour at high altitudes was uncertain. Similarly the U-tube fatigue test gave an average duration of 57·3″ in the first class, of 38·7″ in the second, and of 47·4″ in the third, so that those whose behaviour was uncertain come roughly midway between the good and bad types (except for the maximum expiratory force which appears to have been slightly lowered by fatigue in the good type). This, too, in spite of the fact that the average number of hours flown was 240 hours in the good, 131 in the bad, and 175 in the uncertain type. Though fatigue has undoubtedly lowered the figures obtained in many cases, yet it must mask rather than cause the contrast observed, since there can be no doubt that the manometer results have been lowered in the good type by fatigue, so that originally their average must have been very high. Also it is noticeable that the average figures obtained even in the bad class are only very slightly below the Commissions Board minimum standards, and that only in the fatigue test. Of the good type, 34·6 per cent. are below the minimum standard expiratory force of the Commissions Board

TABLES

TABLE I. GOOD AT ALTITUDES

No. and Name.	*History*	*Flack's Manometer.* *Max.*	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M. V. C.*	*Factor.*	*Remarks*
1. H. C. B.	Good by Dreyer's test and on mountains. No high flying. Dreyer test (No. 5).	120	85″	5375	8750	16	546	1·63	26	
2. J. B.	80 hrs. Boarded P. U. F. Dec. 17. Tested May 1918 by Dreyer's method and found fit to fly below 10,000 ft. (Dreyer, No. 37.)	100	68″	3850	7450	13	570	1·9	25	Limited to 10,000 ft. owing to his old history of cardio-vascular debility. Fit at 18,000 during test.
3. G. D.	156 hrs., Bristols. Tired. Has educated himself to deep breathing at altitudes, finding it useful.	198	60″	3600	8800	10	880	2·45	25	Evacuated to England for rest and H.E.*
4. D. C.	No high flying. Good at altitudes when tested by Flack's bag.	140	120″	5500	6250	15	416	1·14	17	
5. H. E.	105 hrs. on day bombers. Recently concussed.	75	40″	3700	5500	10	550	1·5	15	Respiratory rate without tube, 11. Sent to convalescent hospital.
6. D. A. S.	120 hrs. on Bristols. Fracture of carpus—otherwise fit.	85	44″	3900	5600	16	350	1·4	22	Evacuated to England.
7. J. L. B.	M. O. but has proved to be very good at high altitudes.	80	69″	4950	6000	10	600	1·21	12	

* H.E. indicates for duty on Home Establishment in England.

TABLE I. GOOD AT ALTITUDES (*continued*)

No. and Name.	*History.*	*Flack's Manometer.* Max.	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M. / V. C.*	*Factor.*	*Remarks.*
8. R. Y. I.	450 hrs. day bombing. Admitted to hospital for debility after influenza. Very good type but pulled down by influenza.	75	65″	5100	8600	22	390	1·7	37	Discharged to duty after one week. Very excitable type. Duty at his own request. Posted as missing a week or so later.
9. J. T. J.	360 hrs., Scouts. Very good type. 35 Huns. Admitted for debility after influenza.	115	50″	3200	5917	18	328	1·8	32	Discharged to duty after ten days at his own request.
10. J. T. J.	Readmitted 18 days after discharge (see above), having had 50 hrs. in the air and 14 Huns since discharge. Very tired after flying, dreaming of flying, and walking in his sleep. Some loss of weight.	90	38″	3200	7100	16	433	2·2	35	Evacuated to England for rest and H.E.
11. D. R. D.	90 hrs., 13 in France. Scouts. Is all right at 16,000 ft., but has trouble with his ears. Has Eustachian obstruction and deflected septum.	95	62″	4700	7000	13·5	518	1·5	20	Evacuated to England for treatment.
12. P. J. C.	450 hrs., Scouts. Rather tired. Feels drowsy after 30 mins. above 18,000 ft. but otherwise does not mind altitude.	70	80″	4000	(3400)	11	(310)	?	?	Ventilating figure probably wrong due to a leaky valve. Evacuated to England for H.E.
13. E. A. C.	398 hrs., day bombers. Due for H. E. Rather tired. Average height only 15,000 ft.	150	63″	4500	10,000	22	450	2·2	48	Evacuated to England for rest and H.E.

14. J. R. C.	212 hrs., Scouts. To hospital for scabies. Rather tired.	95	63″	4750	7250	15	483	1·5	22	Treatment, then sick leave.
15. W. B. I.	213 hrs., Bristols, as observer. Rather tired. Due H. E.	70	45″	3600	6500	12	542	1·8	22	Evacuated to England for rest and H.E.
16. W. J. G.	300 hrs. Scouts. A little stale. Has signs of fatigue.	85	35″	3730	6150	10	615	1·65	17	Evacuated to England and recommended for Canadian leave.
17. C. M. H.	233 hrs., day bombers. Feels stale and has signs of considerable fatigue.	115	60″	4800	5625	11·5	487	1·17	14	Evacuated to England for rest and H.E.
18. W. C. L.	300 hrs. Scouts. Recent repeated vomiting due to Eustachian obstruction. Very tired in consequence.	60	29″	4200	7660	15	510	1·82	27	Evacuated to England for treatment of ears.
19. R. C.	46 hrs., day bombers. Very fatigued on landing. Considered by squadron as temperamentally unsuited to active-service flying.	80	64″	3400	5000	11	454	1·47	16	To duty on ground, temperamentally unfit.
20. S. H. H.	290 hrs., day bombers. Flying 15–20,000 ft. without oxygen. Recent headache. Very tired.	70	47″	3900	5250	5·5	955	1·35	8	Respiratory rate without tube 15 and very shallow. Evacuated to England for rest and H.E.
21. C. L. L.	550 hrs., Scouts. Rather sleepy at altitudes. Very tired.	65	27″	4500	8800	20·5	430	1·95	40	Evacuated to England for rest and H.E.
22. G. S.	110 hrs., day bombing. Cannot keep formation. Astigmatism. Tired after flying at 18,000 ft., but has no other symptoms. Tested Dreyer up to 24,000 ft. Very good.	110	57″	4900	7100	15·5	458	1·45	22	Prefers to transfer to infantry.

TABLE I. GOOD AT ALTITUDES (*continued*)

No. and Name.	*History.*	*Flack's Manometer. Max.*	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M. / V. C.*	*Factor.*	*Remarks.*
23. L. H. T.	170 hrs., Bristols. Recently crashed in flames. Sleeping badly since. Slight tremor.	95	50″	4750	10,500	12·5	840	2·21	28	Evacuated to England for rest and H.E.
24. C. R.	330 hrs. Long distance reconnaissance. Flying 18,000 ft. with oxygen, but has been fairly fit even when this has failed. Losing weight lately. Tired. Due for H. E.	70	58″	4300	7350	13·5	543	1·71	23	Evacuated to England for rest and H.E.
25. W. H. B.	273 hrs., Scouts. Quite fit at altitudes. Tired, with exaggerated reflexes and tremor.	115	54″	3550	6850	17·5	390	1·93	34	Evacuated to England for rest and H.E.
26. J. H. P.	620 hrs., Bristols. Recently slight sleepiness in the air. Highest flown, 20,000 ft.	75	55″	4600	5000	14·5	345	1·09	16	Evacuated to England for rest and H.E.
	AVERAGES	96	57·3″	4260	6970	14·1	523	1·55	24·1	
	Below Standard (minimum U-tube)	34·6%	15·4%	4%		15·4%		20%	24%	
	Below Average Standard U-tube	69·3%	38·5%							

Passing all tests, 46·2%.
Average number of hours flown, 240.

TABLE II. BAD AT ALTITUDES

No. and Name.	*History*	*Flack's Manometer.* *Max.*	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M V. C.*	*Factor.*	*Remarks.*
1. R. L. T.	51 hrs. (1½ in France). Ill above 13,000 ft. Tachycardia and respiratory rate above 30 even in bed.	90	35″	?	11,250	44	255	?	?	D.A.H. type. Evacuated to England.
2. F. R. G.	42 hrs. Scouts. Dizzy above 10,000 ft. Tested by Dreyer (38)—rather bad. Cheynes-Stokes type.	170	48″	3200	9280	17	530	2·85	48	Only moderately bad. To try again and use O_2 if necessary.
3. C. R. B.	240 hrs. Scouts. Cannot stand over 10,000 ft. Very bad by Dreyer's test. Excessive respiratory type (Dreyer test 36).	30	20″ (to 20 mm. only)	2800	11,500	15·5	745	4·2	65	To fly below 10,000 ft.
4. F. F.	700 hrs. flying. Corps work. Tired. Highest flown 16,000, feels very ill above 15,000 ft.	115	45″	3900	8750	20·5	440	2·2	45	Evacuated to England for H.E.
5. H. J. E.	597 hrs. Scouts. Recently sleepiness and dyspnoea at 18,000 ft. Not really comfortable above 10,000 ft. now. Very tired.	80	40″	4000	10,550	16·5	640	2·6	43	Evacuated to England and recommended for Australian leave.
6. N. M. S.	145 hrs. (25 in France). Faintness above 14,000 ft. Scouts. Tested by tube method and found to be rather bad.	75	45″	4050	6250	18	345	1·5	27	Not very bad. Physical training and then returned to duty.

TABLE II. BAD AT ALTITUDES (*continued*)

No. and Name.	*History.*	*Flack's Manometer. Max.*	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M. / V. C.*	*Factor.*	*Remarks.*
7. C. F. C.	Scouts. Hours uncertain. Age 38. Abdominal pain at high altitudes. (Query, 100 hrs.)	130	45″	4900	11,350	14·5	785	2·3	33	Only moderately bad. Returned to duty at own request.
8. C. P. T.	100 hrs. Highest flown 6000 ft. Tested by tube method, not at all good. Subnormal vascular response with fall of blood pressure.	?	?	3725	7750	28	276	2·1	59	Respiratory rate without tube = 16.
9. J. A. B.	130 hrs. (20 in France). Fainted in air at 12,000 ft. On day bombers. Only 20 hrs. of high flying. Tested tube method. Subnormal vascular response and Cheyne-Stokes respiration.	65	30″	3700	8100	15·5	520	2·9	45	To cease flying. Temperamentally unfit.
10. L. P. K.	140 hrs. (20 in France). Day bombing. Fainted at 10,000 ft. in the air. Tested tube method 14,000 ft. Bad periodic breathing, pain in praecordium, and commencing heart failure.	60	30″	3850	6750	12	563	1·75	21	To fly night bombers only. Very good type.
11. C. P.	20 hrs. as observer day bombers. Headache above 10,000 ft. with impairment of judgement.	50	25″	3325	12,250	26	480	3·7	96	To cease flying. Temperamentally unfit.
12. R. N. W.	50 hrs. (none on active service). Felt faint at 5000 ft., and was ill on landing. Has fainted on the ground. Good type but signs of cardio-vascular debility.	40	20″	3300	5600	14	400	1·7	24	To cease flying. Temperamentally unfit.
13. A. M.	170 hrs. day bombing. Above 15,000 ft. feels sick and giddy, has vomited. Headache.	120	45″	4000	9000	23	390	2·25	52	To do ground work.

14. J. W.	100 hrs. (30 in France) day bombing. Feels bad above 9000 ft. and cannot get his breath. Tested by Dreyer method. Very bad. Periodic respiration (Dreyer 40).	50	33″	4400	9100	24	380	2·1	50	**Evacuated to England.** Quite unfit for high flying.
15. W. C. F.	54 hrs. (3 in France). Feels ill even at 9,000 ft. Dreyer test (41) showed he was very bad with excessive respiratory response and dyspnoea.	55	37″	3300	6200 ↓ (11,250)	26 ↓ (34)	230 ↓ (331)	1·9 ↓ (3·4)	49 ↓ (115)	Big increase in ventilation during test. Respiration very unstable. Evacuated to England being unfit for general service.
16. J. H. G.	100 hrs. (50 in France) on day bombers. Highest flown 14,000 ft. Feeling ill lately and fainted in air before admission to hospital. Tested by Dreyer methods—very bad, with subnormal response (Dreyer 43).	70	60″	3725	12,600	20	630	3·4	68	To fly night bombers only.
17. A. M. B.	80 hrs. (10 in France). Giddy above 16,000 ft.	50	29″ (25 mm. only)	3200	5500	30	183	1·7	51	Ventilation figure uncertain. Little short puffing respirations. Respiration at rest 22 without tube. Boarded P.U.F.* Fit for general service.
18. W. H. G.	99 hrs. (5 in France). Feels dizzy if above 13,000 ft. Scouts. Tested by Dreyer (45). Fainted after having very periodic breathing.	105	65″	4300	7150	14	510	1·7	24	Evacuated to England for treatment of Eustachian obstruction, which he also had. No use for scouts.

* P.U.F. indicates permanently unfit for flying on medical grounds.

TABLE II. BAD AT ALTITUDES (*continued*)

No. and Name.	*History.*	*Flack's Manometer.* *Max.*	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M. / V. C.*	*Factor.*	*Remarks.*
19. A. S. M.	141 hrs. (50 in France). Scouts. Dyspnoea above 11,000 ft. Tested by Dreyer (25). Later developed height psychosis.	120	53″	4700	6610	12·5	527	1·4	18	Transferred to infantry at his own request.
20. N. M. D.	100 hrs. Observer on Bristols. Short of breath above 18,000 ft. Had rapid breathing and gasping for breath. This got worse until he could not go into the air at all.	95	35″	3280	7100	23	308	2·2	1	Has done six weeks' work on the ground and improved. To do three months' more ground work. Useless or high flying.
21. J. K. G.	60 hrs. (5 in France). Scouts. Never higher than 9500 ft. when he feels uncomfortable. Tested on Dreyer, considerable distress with periodic breathing at 16,000 ft. (Dreyer 46).	75	45″	3600	7316	15	490	2·04	31	To fly below 10,000 ft. To seaplanes.
22. L. D. R.	78 hrs. Nil in France. Scouts. Bad crash when training. Giddy above 5000 ft. Tested Dreyer 16,000 ft. not good. Has low diastolic pressure.	70	40″	3750	4750	10	475	1·25	13	Ventilation figures probably inaccurate with leaking valve. Evacuated to England.
23. J. B. P.	40 hrs. Nil in France. Crashed when training. Dizzy above 10,000 ft. Tested by Dreyer (47) to 16,000 ft. Periodic breathing and bad response.	80	48″	4600	5375	11	488	1·15	13	Disability probably largely temperamental. Periodic type of bad response. To duty on ground for temperamental reasons.

24. G. H. D.	19 hrs. Observer on day bombers. Long period with infantry with concussion. Feels faint at 13,000 ft. Tested by Dreyer to 16,000 ft. Bad with periodic breathing.	70	40″	4100	10,250	23	446	2·5	57	To fly below 4000 ft.
25. J. C. W.	106 hrs. day bombers. Headache and dizziness if doing a long period above 15,000 ft.	85	22″	3750	7600	14·5	524	2·02	29	Only moderately bad at altitudes. To fly night bombers.
26. S. S. G.	17 hrs. as observer on day bombers. Tested Dreyer to 18,000 ft. Increase to respiratory rate to 52 with headache and sleepiness.	130	50″	3100	9900	28	353	3·2	90	Very bad at present. Evacuated to England for physical training and later change to low-flying machines.
27. W. B.	110 hrs. day bombing. Cannot fly straight above 10,000 ft. Feels very sleepy and observers complain that he is inefficient at these altitudes.	75	37″	3700	10,620	15·5	688	2·87	45	To duty on night bombers only.
28. W. J. B.	85 hrs. (5 in France) day bombing Feels ill over 5000 ft. D. A. H. type of pulse.	50	23″	3500	9500	38	250	2·71	103	Respiratory rate without tube 22. Passed P.U.F.
	AVERAGES	81·8	38·7″	3770	8500	20·1	452	2·3	46·3	
	Below Standard (minimum U-tube)	55·5%	48·2%	29·6%		57·2%		64·3%	71·5%	
	Below Average Standard U-tube	74·1%	89%							

Passing all Tests, 10·7%.

Average number of hours flown, 131.

TABLE III. UNCERTAIN AT ALTITUDES

No. and Name.	*History.*	*Flack's Manometer.* *Max.*	*Duration at 40 mm.*	*Vital Capacity*	*Ventilation per minute.*	*Respiratory Rate.*	*Tidal Air.*	*V. M. / V. C.*	*Factor.*	*Remarks.*
1. A. H. R.	90 hrs. night bombing. Hospital for influenza. Has never been to high altitudes.	140	45″	4650	8600	7	1255	1·85	13	Respiration much slowed by tubes and valve. Sick leave.
2. S. W. K.	120 hrs. Observer on Bristols. Good, but recently tired and has eye trouble, apparently due to fatigue.	120	43″	3900	10,800	21	510	·2	67	Evacuated to England for rest and H.E.
3. W. H.	462 hrs. Corps work. No high flying. Tired and due for H.E. Age 40.	120	44″	3500	8920	11	812	2·55	28	Evacuated to England for rest and H.E.
4. R. J. W.	64 hrs. (none in France). Has never been above 6000 ft. Feels lonely. Height Psychosis. Wants to do low strafing.	75	32″	3650	7000	13	538	1·9	25	To medical flight.
5. J. E. B.	197 hrs. day bombing. Continuous headache after 47 hrs. high flying. No other cause found.	85	24″	3700	7800	20	390	2·1	2	To do ground work at present.
6. E. B. O. B.	184 hrs. (24 in France). Short of breath and giddy above 10,000 ft. Bristols. Tested on Dreyer (44) to 20,000 ft. and was very fit but had very rapid respiration. Is very unlikely to remain good at high altitudes.	115	58″	3600	13,250	22	615	3·75	82	Though at present fairly fit not a type suited to high flying. Discharged fit for duty, now working as ferry pilot. Very excitable subject.

7. A. E. H.	210 hrs. on day bombers. Always flies using oxygen and has a very bad headache if this fails. Now very tired.	100	1″	4400	9100	13	700	2·1	27	Probably fairly good at height but not better than that. Evacuated to England for rest and H.E.
8. P. H. D.	194 hrs. as observer in Corps work. No high flying. Due for H.E. Tired.	75	45″	4500	7500	12	625	1·7	20	Evacuated to England for rest and H.E.
9. D. S. G.	25 hrs. on balloons. Old depressed fracture of skull without operation. Symptoms of traumatic neurasthenia. Headache. Accident 18 months before examination.	55	33″	3250	6250	11	580	1·9	21	Fast unstable pulse of D.A.H. type. Evacuated to England for discharge from Army.
10. V. G. C.	75 hrs. Scouts. Recent severe concussion. Has been to 18,000 ft. and been quite fit. Headaches since crash.	75	48″	3100	10,750	2	450	3·5	84	Unstable pulse of mild D.A.H. type. Evacuated to England.
11. A. F. W.	200 hrs. Observer in Corps work and night bombing. Has never been to high altitudes. Due H.E.	300	96″	4000	12,500	16	735	3·1	50	Evacuated to England for H.E.
12. D. W. F.	580 hrs. Scouts. Patrols at 16,000 ft. make him sleepy. Recently has repeatedly vomited in the air. Very tired.	84	38″	4575	10,375	14	740	2·3	32	Evacuated to England for rest and H.E.
13. J. E. B.	70 hrs. flying. Dyspnoea and vomiting above 10,000 ft. On day bombing. Tested by tube method and found fairly good. Has deflected septum and some ear trouble.	100	50″	3300	6250	9·6	650	1·9	18	? due to oxygen want or trouble with ears. To medical flight.

TABLE III. UNCERTAIN AT ALTITUDES (*continued*)

No. and Name.	*History.*	*Flack's Manometer. Max.*	*Duration at 40 mm.*	*Vital Capacity.*	*Ventilation per minute.*	*Respiratory Rate*	*Tidal Air.*	*V. M. / V. C.*	*Factor.*	*Remarks.*
14. H. C. B.	140 hrs. day bombing. Always uses oxygen. Once did a 3-hr. show at 18,000 ft. without oxygen. Had to go to bed afterwards.	95	60″	3900	7600	19	400	1·95	37	In hospital following a crash. Probably would never last at high altitudes without oxygen.
15. C. N.	90 hrs. (10 in France). Day bombing. Felt queer at 16,000 ft.	80	47″	4200	6250	15·5	405	1·4	22	Immature youth, with low blood pressure. To fly, using oxygen.
16. W.	M. O.	100	60″	3500	9000	12	750	2·57	31	
17. A. D. M.	113 hrs. (2 in France). Faints at the sight of blood. Corps work. Highest flown 13,000. Not very fit at this altitude.	70	57″	3950	5250	11·5	362	1·35	16	To duty.
18. C. C. W.	330 hrs. night bombing. Rather tired. Would like to do work on scouts.	60	25″	3750	5000	17	295	1·33	23	Evacuated to England for rest and H.E.
19. C. W.	118 hrs. Bristols. Sleeping badly last three weeks of his time, then wounded a month before examination. No flying since he was wounded.	70	35″	3650	9000	25	360	2·46	61	Evacuated to England for rest and H.E. Probably would never stand much high flying.
	AVERAGES	100·8	47·4	3850	8500	15·4	588	2·26	36·8	
	Below Standard (minimum U-tube)	36·8%	31·6%	15·8%		36·8%		47·3%	47·3%	
	Below Average Standard U-tube	73·7%	68·4%							

Passing all tests, 26·3%.

Average number of hours flown, 175.

(80 mm.) and 15·4 per cent. below the minimum standard of 40 mm. for the fatigue test, a low percentage considering the number of hours flown and the resulting fatigue.

On the other hand, of the bad type 55·5 per cent. are below the minimum standard expiratory force, and 48·2 per cent. below the minimum standard for the fatigue test, while in the uncertain group 36·8 per cent. are below in the former, and 31·6 per cent. in the latter test.

The low figures obtained in the bad types even after very little flying may perhaps be due to their flying experiences; some of these patients have fainted in the air, and such experiences seem to reduce the vitality of the individual to a low level. Thus one officer with 67 hours flying who had fainted in the air on his last trip was only able to blow the manometer to 45 mm. five days later, while after ten days he could blow it to 55 mm., after eighteen 75 mm., and after 22 days 90 mm., and in the same time he improved his maintenance of the mercury at 40 mm. from 30″ to 38″. It is, therefore, unlikely that any excessively low figures would be found in these officers before they had experienced fatigue or symptoms due to high altitudes. None the less those giving results much above the minimum standards would probably last better on high-flying machines than those barely passing these standards, and there can be little doubt that these tests are of value in the selection of men for flying at high altitudes, particularly if the average standards for the tests be taken and not the minimum. (Average standards 105 mm. and 52″.)

Vital Capacity. A minimum vital capacity of 3,000 c.c. has now been fixed by the Commissions Board. This standard appears to take no account of the size of the individual. For high flying it is suggested that no one with a capacity below 3,400 c.c. be accepted unless he can hold his breath for more than 45 secs. It will be noticed that in the good type there is included one excellent scout pilot (Table I, 9), with a vital capacity of 3,200 c.c., he being a very small man. It will be seen, however, that he can hold his breath 50 secs. This officer has thirty-nine enemy machines to his credit. Taken as a whole, however, only 4 per cent. of the good type are below the 3,400 c.c. standard, and the average value is 4,260 c.c., while in the bad type 29·6 per cent. are below this standard, and the average is 3,770. As before, the uncertain group takes an intermediate place with an average of 3,850 c.c. The vital capacity test is therefore also valuable, especially if considered in relationship with the size of the individual, or better with his normal air consumption per minute.

The ventilation per minute shows a higher figure for the bad type than for the good—a consumption of 8,500 c.c. as against 6,970 c.c., and this seems to be partly dependent on the existence of a fast shallow respiration, but not entirely so. Others have shown how a high-air consumption is found associated with the tachycardia in a D.A.H. condition, and a similar large consumption of air is found in the thin anxious type of individual. In the tired pilot who is beginning to be nervous, to be tremulous, to have exaggerated reflexes and to sleep badly, a history of loss of weight

is quite common, and in these cases also the air consumption appears to be raised quite apart from any increase in the respiratory rate. A good instance may be seen in Table I, 9 and 10, a case which also serves to illustrate the changes seen with the U-tube manometer as the result of fatigue. It appears that these fatigue results, though aggravated by high altitude flying, are more due to the nervous strain of fighting than to flying itself (cf. cases included in Table III).

The respiratory rate is 14·1 per minute in the good type, 20·1 in the bad, and 15·4 in those whose behaviour at altitudes is uncertain. If a standard of 16 per minute is taken then 23 per cent. have a rate faster than this in the good, 57·2 per cent. in the bad, and 36·8 per cent. in the uncertain type. A slow respiration is therefore a good sign, and if it is still further slowed on meeting slight resistance to expiration so much the better. The respiration rate was rather unusually high in the patients recorded in the Table I, 8 and 9 considering their flying history, but they had both recently had influenza, and it is possible that this disease tends to quicken the respiratory rate in a way similar to its effect on the pulse-rate.

The tidal air is smaller in the bad types than in the good, since their respiration is generally quicker and shallower, but many very small tidal airs are also found in the good types in whom the ventilation figure is low, and in this case, when associated with a slow respiration rate, it is no disadvantage.

The ratio of the ventilation per minute to vital capacity seems of considerable importance, and this has been expressed by recording the value $\frac{\text{ventilation per minute}}{\text{vital capacity}}$. It is obvious that if an individual requires a large amount of air per minute he will require a large vital capacity also, if he is to stand high altitudes well, and this has been found to be the case. In the good type this ratio has an average value of 1·55, in the bad 2·3, and in the uncertain group of 2·26 ; in the first class only 20 per cent. have a value above 2·0, in the second 64·3 per cent., and in the last 47·3 per cent. It would appear, therefore, that the value of vital capacity tests is much greater, if the vital capacity be judged according to the relationship it bears to the air consumption of the individual.

Lastly, an *artificial factor* has been deduced which seems to give some idea of the ability of the individual to stand high altitudes from the respiratory point of view. Since a slow respiration, a large vital capacity, and a low ventilation rate all indicate an increased reserve for use at high altitudes, this factor consists simply of the value $\frac{\text{respiratory rate} \times \text{ventilation per minute}}{\text{vital capacity}}$, and a low figure indicates a good type. A figure of 30 has been taken as the standard, those above this being relatively bad.

It will be seen that the average figure for the good type is 24·1, for the bad 46·3, and for the uncertain 36·8. Similarly, 24 per cent. of the good type fall above 30, 71·5 per cent. of the bad, and 47·3 per cent. of the uncertain. This factor seems, therefore, to be even more effective at selecting those bad at altitudes than the U-tube manometer, and though it too may be influenced by fatigue, it is

probably more constant in any individual than the results obtained with the U-tube fatigue test.

If, now, the results with the U-manometer and this factor be alone considered, it will be seen that in the good type, containing many tired men (the average hours flown being 240), 46·2 per cent. pass all three tests, 57·7 per cent. pass both U-tube minimum standards, and 76 per cent. pass on the factor standard. In the bad type (the average number of hours being 131) only 10·7 per cent. pass all tests, 33·3 per cent. pass both U-tube minimum standards, and 28·5 per cent. pass the factor standard. In those uncertain at altitudes (average number of hours flown being 175), 26·3 per cent. pass all three tests, 52·7 per cent. pass both U-tube minimum tests, and 52·7 per cent. pass the factor standard. It is clear that the factor standard often picks out cases which pass the U-tube tests (minimum standard) and vice versa, but the factor seems to be more efficacious at picking out those bad at altitudes than the two manometer tests combined when the minimum standards are employed.

The average standards of the U-tube test are much above the level attained by most of those in Group II, 74·1 per cent. being below the maximum average standard and 89 per cent. below the average figure for fatigue. Yet this test shows such variations from day to day, according to the temporary physical condition of the patient, that it is unlikely to prove as valuable as simple respiratory measurements in the selection of airmen for high flying. In this connexion it is noticeable what a large proportion of the good type, when they are tired, also fall below these average standards.

C. The Value of the Methods employed.

The U-manometer test as designed by Flack has proved its value as a test of physical fitness of the individual at the time of examination, and for this purpose the other tests here described have little importance. No very definite conclusions can be drawn from figures obtained in pilots in various stages of exhaustion, but it is probable that by some such examination of the vital capacity, ventilation per minute, and respiratory rate, it should be possible to pick out those airmen whose physique makes them peculiarly suitable for high altitude work, especially as a large percentage of pilots are able to stand altitudes fairly well. It would be wise to take into consideration the U-tube results at the same time, but it should be recognized that of the two tests the U-tube tests are probably less important. Even if these tests were employed before training pilots on high-flying machines, a certain number would still eventually be found unable to stand the high altitudes, since cardio-vascular failure may also occur, as well as a periodic type of respiration sufficiently severe to cause inefficiency. But, if the effects of fatigue be allowed for in the results here recorded, it seems probable that the wasteful training of men unsuited for high flying might be avoided in from 70 per cent. to 80 per cent. of the cases, if these simple respiratory tests were employed before the

men were trained for this type of work, and, if those who showed low diastolic pressures were also not allowed to attempt flying at high altitudes, any such wasteful training might perhaps be reduced still further.

D. Conclusions.

(1) That men with a high vital capacity, a low consumption of air per minute, and a slow respiratory rate, have a greater reserve than others to enable them to withstand high altitudes.

(2) That this extra reserve may be represented roughly by a factor $\frac{\text{respiration rate} \times \text{ventilation per minute}}{\text{vital capacity}}$, and that any figure thus obtained below 30 is good, and above this poor.

(3) That additional evidence of good types of physique for high altitude flying may be obtained from the results with the U-tube manometer tests, but that these are less important from this point of view. They are more affected by fatigue than the former results, and especially by the fatigue of great nervous strain, so that their value in measuring temporary exhaustion is very great.

(4) That by the employment of these simple tests early in the training of a pilot or observer, it might be possible to select those likely to be able to stand altitude well, and to abolish 70 per cent. to 80 per cent. of the failures at altitudes that now occur, so eliminating much wasteful and expensive training.

Note.—I am indebted to Mrs. Briscoe for a personal communication, before these observations were commenced, in which she pointed out that the oxygen consumption per minute was often above normal in pilots who were hospital patients. Her observations, though viewed from a different aspect, would appear to agree with the rough empirical tests here reported.

Appendix.

In revising this paper for republication reference may be made to the recent interesting paper by Dreyer (1) in which he correlates vital capacity with weight and other measurements. No calculations of this character are possible with the material here collected, since no means were available for determining the exact weight of the subjects examined. Since the metabolism has been shown to bear a similar relation to the surface area (2), the comparison of vital capacity with the ventilation rate is a similar process. For the purpose of estimating the capacity of an individual to withstand high altitudes I consider the method here adopted the more useful, since there is no guarantee, especially under war conditions (cf. Mrs. Briscoe's results), that the metabolism is at a normal level for the surface area of the individual, while the relationship of the vital capacity to the work it has to do remains the factor of primary importance. That big variations in the vital capacity occur with variations in physical fitness, as suggested by Dreyer's paper, I can confirm in that my own vital capacity has varied between 5,375 c.c. (Table I. 1) and 4,500 c.c. during the last 18 months.

References.

(1) G. Dreyer, *Lancet*, Aug. 9, 1919, p. 227.
(2) Atwater and Benedict, 1903. J. C. Aub and E. Du Bois, *Cornell Univ. Med. Bulletin*, vii, 1918

10.—OBSERVATIONS ON THE RESPIRATION OF AIRMEN

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THESE observations on the respiration of airmen admitted to hospital for various causes were carried out by means of the apparatus devised by Douglas for studying the respiratory exchange.

The object of the investigation was to see if any marked deviation from the normal could be detected in the respiratory mechanism of airmen suffering from one form or other of flying disability.

A. OXYGEN METABOLISM.

The apparatus for determining the total respiratory exchange consists of a large rubber canvas-covered bag, containing 30 to 40 litres when moderately distended. This bag is connected by a rubber tube of large calibre with a mask, which covers the mouth and nose, and which is provided with inspiratory and expiratory valves. Between the mask and the bag is a three-way tap of large bore, so that the subject can breathe into the bag or into the open air at will. The subject sits comfortably in an arm-chair, with the mask pressed closely over the nose and mouth. The bag is fixed behind the chair, so that he is unable to see the amount of distension. With each breath he takes in fresh air through the inspiratory valve and all the expired air passes out through the expiratory valve and tube. For the first two minutes the tap in the connecting tube is turned, so that the expired air passes out into the room, and when the patient is quite comfortable with the mask, the tap is turned, so that all the expired air passes into the bag. The expired air is collected over a timed period (3 to 5 minutes according to the amount of lung ventilation) and the rate of respiration noted. At the end of the timed period, the tap is turned and the rubber tube disconnected from the expiratory valve and attached to a gas meter. While the air is being passed through the meter, a sample is taken through a small by-pass connected with the bag. The composition of the air in the sample is analysed, and this analysis gives the percentages of oxygen and carbon dioxide in the expired air. The amount of oxygen used by the individual is calculated from the difference in the percentages of oxygen in the inspired and expired air, and the amount of air which passes through the lungs per minute.

In order to compare subjects of varying weight, the actual amount of oxygen consumed has been divided by the weight of the individual in kilograms and the figure thus obtained, i. e. the amount of oxygen consumed per minute per kilogram has been taken as the standard of comparison.

As far as possible all the patients were examined under the same conditions. It was not possible to carry out the investigation under conditions of complete rest, but the examination was made early in the day, between 10 and 12 a.m., and after sitting comfortably at rest in an arm-chair for about a quarter of an hour.

As individuals vary from day to day in the rate of oxygen usage, the first cases were examined at some length, to get an idea of the amount of variation. In some patients the rate from day to day was very constant, in others the variation was considerable, and seemed to be independent of small changes in temperature and pressure. In Table I the figures obtained in two cases are given in detail; in No. 1 the rate of consumption is practically the same on three different days, in No. 2 the figure varies by about 14 per cent. In the case of No. 1, the room temperature varied by 6° F., and in the case of No. 2 by 1° F.

The results obtained in a series of 30 pilots and observers are given in Table II. Where more than one observation has been made, average results are given. The cases are given in the table in the order in which they were examined, without any kind of selection. They include some fairly fit men on the point of being discharged from hospital, and some cases with marked nervous symptoms. The figures given may be compared with those obtained from a series of controls (Table III). This series of controls includes four fit experienced airmen and eight flying cadets, just passed as fit by the Commissions Board. The patients were examined in the morning before they had left the hospital or taken any exercise; the controls all had to come in from outside the hospital, and the successful airmen had a motor drive from a neighbouring aerodrome. Under these conditions the rate of metabolism at rest should have been lower in the hospital patients than in the controls.

The average oxygen consumption (measured at room temperature) for the 12 controls is 4·7 c.c. per min. per kilo (minimum 3·9 c.c., maximum 5·3 c.c.), the average figure for the thirty patients is 5·1 c.c. per min. per kilo (minimum 4·4 c.c., maximum 6·1 c.c.). The majority of the patients showed definite symptoms of a nervous nature. If the cases (18 in number) classed clinically as 'neurasthenics' be taken separately, the average rate of oxygen consumption is 5·3 c.c. per min. per kilo, while the average rate in the remaining cases (12 in number) is 4·9 c.c. per min. per kilo. Taken as a whole the higher figures were obtained in 'nervy' patients of the jumpy restless type.

It is difficult to compare these figures with those obtained by other methods, as most of the results recorded refer to oxygen consumption at complete rest, i. e. lying down and with complete muscular relaxation.

Benedict gives the following figure as an average of daily estimations for 3 months on an athlete, at complete rest and on a regular

TABLE I

Name.	*Date.*	*Litres of air per min.*	*Breaths per min.*	*Vol. of each breath, in c.c.*	% O_2 *expired air.*	% CO_2 *expired air.*	*Alveolar* CO_2 %	*Measured moist and at prevailing barometric pressures.* O_2 *consumed per min. in c.c.*	CO_2 *expired per min. in c.c.*	O_2 *consumed per min. per kilo., in c.c.*	*Dead space, in c.c.*
Lt. G. :											
Weight, 64·3 kg.	27/2/18	6·4	12·5	516	16·33	3·85	5·77	296	246	4·6	119
Hours flying, 160.	1/3/18	7·85	16·5	478	17·01	3·57	5·55	308	280	4·7	121
	2/3/18	7·5	16	468	16·97	3·54	5·66	300	265	4·6	123
Lt. B. :											
Weight, 63·8 kg.	6/3/18	6·25	18	347	16·44	3·77	6·07	283	235	4·4	64
Hours flying, 100.	7/3/18	7·02	19	369	16·38	3·69	6·63	321	259	5·0	110
	9/3/18	6·9	18·5	372	16·47	3·75	6·09	310	258	4·8	91

TABLE II

Name.	Hours flying.	Weight in kg.	No. of observations.	Litres of air per min.	% O_2 in expired air.	% CO_2 expired air.	*Measured moist at prevailing baro. pressures.* O_2 consumed per min. in c.c.	CO_2 expired per min. in c.c.	O_2 consumed per min. per kilo, in c.c.
Lt. A. H. W.	45	73·0	4	8·6	16·31	4·09	398	351	5·4
Lt. F. W.	13	54·3	5	6·1	16·53	4·01	270	245	4·9
Lt. W. D.	100	67·0	5	5·3	15·61	4·83	294	261	4·4
Lt. E. M. C.	6	66·2	4	9·5	17·28	3·35	361	335	5·4
Lt. J. C.	160	68·4	3	8·2	16·97	3·26	328	267	4·8
Lt. A.	1,575	69·8	3	9·8	17·64	2·94	327	288	4·7
Lt. B.	100	55·3	3	8·0	17·02	3·48	314	277	5·7
Capt. S.	—	60·7	2	8·4	17·03	3·29	331	277	5·4
Lt. H.	—	63·8	1	8·2	16·9	3·38	330	277	5·1
Capt. H.	600	61·6	3	6·3	16·58	3·37	273	212	4·4
Lt. B.	100	63·8	3	6·7	16·43	3·73	304	250	4·7
Lt. A. G. G.	160	64·3	3	7·2	16·77	3·65	301	263	4·6
Lt. C.	167	53·5	3	8·0	17·45	3·51	283	280	5·2
Lt. C.	50	56·2	2	6·9	17·16	3·26	262	230	4·6
Lt. M.	—	65·2	1	6·6	16·56	3·66	291	241	4·5
Capt. L.	180	69·7	4	8·8	16·87	3·56	357	313	5·1
Lt. V.	200	56·2	3	9·6	17·3	3·5	348	336	6·1
Lt. M.	400	62·0	2	5·9	16·02	4·04	292	240	4·7
Lt. W. J. W.	300	55·8	4	8·4	17·26	2·8	308	235	5·5
Lt. B.	13	67·1	2	9·6	16·73	3·36	403	322	6·0
Lt. G. H. B.	27	52·1	1	6·5	16·4	3·4	298	223	5·7
Lt. C.	18	76·2	1	9·1	16·24	3·22	429	295	5·6
Lt. L.	100	57·1	1	7·0	16·94	3·07	282	217	4·9
Lt. C.	400	64·7	2	8·4	17·37	2·87	299	241	4·6
Lt. D.	500	69·7	1	6·2	14·92	3·89	375	243	5·3
Lt. R.	200	63·4	1	8·3	17·2	2·69	309	223	4·9
Lt. M.	55	59·8	1	7·5	16·35	3·9	349	295	5·8
Lt. C.	30	61·6	1	6·6	16·04	3·95	322	260	5·2
Lt. N.	360	57·5	1	5·7	16·18	3·41	271	195	4·7
Lt. F. A. G.	73	58·4	1	6·8	16·58	2·95	298	203	5·1
						Average	..	.. ..	5·1

TABLE III. CONTROLS

	Name.	Hours flying.	Weight in kg.	No. of observations.	Litres of air per min	% O_2 in expired air.	% CO_2 expired air.	*Measured moist at prevailing baro. pressures.* O_2 consumed per min. in c.c.	CO_2 expired per min. in c.c.	O_2 consumed per min. per kilo, in c.c
	Capt. B.	1,000	67·9	1	8·1	17·05	3·36	317	272	4·6
	Lt. W.	600	57·17	1	8·3	17·42	2·90	291	240	5·0
	Lt. C.	600	71·66	1	9·3	16·96	3·91	369	363	5·1
	Capt. K.	600	70·76	1	9·39	17·04	3·56	361	339	5·1
Flying cadets	A. H. C.	—	56·2	1	7·8	17·18	2·77	292	216	5·1
	R. C.	—	72·1	1	7·5	16·47	3·4	334	255	4·6
	Lt. E.	—	61·6	1	6·18	16·51	3·07	273	189	4·4
	L. G. M.	—	67·0	1	6·8	15·92	3·85	344	264	5·1
	J. F. P.	—	65·6	1	8·8	17·56	2·84	296	249	4·5
	J. S.	—	67·0	1	7·6	17·49	2·73	261	207	3·9
	R. B. D. C.	—	68·9	1	8·8	16·75	3·41	367	302	5·3
	A. S.	—	61·0	1	7·8	17·17	3·04	293	237	4·7
							Average	..	.. ..	4·7

diet, 3·6 c.c. (at S. T. P.) per min. per kilo—minimum 3·4 c.c., maximum 3·9 c.c.

The figure given by Geppert for a man at rest is 264 c.c. (at S. T. P.) of oxygen per hour and kilograms. This amount measured at 15° C. 760 mm. would work out at 4·6 c.c. per min. per kilo.

Douglas, using the bag method described above, gives figures which would amount to 3·9 c.c. per min. per kilo at room temperature for a man at rest (lying) and 5·3 c.c. per min. per kilo for rest (standing). The figure for rest (sitting) would lie between these two.

Martin Flack, by the same method as Douglas, gives an average figure of 4·7 c.c. per min. per kilo (measured at room temperature) for rest after sitting quietly in an arm-chair for half an hour.

The figures given indicate a slight but definite rise in the oxygen consumption of patients suffering from one form or other of flying disability. A man weighing 60 kilo will use 15 litres of oxygen per hour at 4·4 c.c. per min. per kilo, while a man of equal weight will use 21 litres of oxygen at 6·1 c.c. per min. per kilo. This difference is not likely to be less, and is probably more, when the muscles are used at high altitudes, and the man with an extravagant resting metabolism is almost certain to be at a disadvantage in a rarefied atmosphere.

Prolonged flying in itself does not appear to produce a rise in the rate of oxygen consumption while at rest. If the patients in Table II are divided into two groups, in which (*a*) the rate of oxygen consumption is between 4·4 c.c. and 5·1 c.c. per min. and (*b*) the rate is above 5·1 c.c. per min., it will be seen that the average number of hours flying is just under 300 in the first group, and just over 120 in the second.

It is worthy of note that, out of six cases giving a history of concussion, five are on the high side, the rate varying between 5·1 c.c. and 6·0 c.c. per min. per kilo.

B. The Rate and Depth of Respiration.

The rate and depth of respiration in a series of patients were determined by the Douglas bag method, as described in Section A; and the results compared with those obtained from a series of fit men examined under the same conditions.

The results are given in Tables IV and V.

The Rate of Respiration.

The average figure for 37 patients is 18·4 per min., the lowest individual figure being 7 per min., and the highest 29·4 per min. The average figure for 16 fit men is 15·2 per min., the lowest 7·3 per min., and the highest 22 per min.

The Volume of Air Breathed.

The average volume of air breathed is practically the same in both series, 7·7 litres per min. in patients and 7·9 litres per min. in the fit men.

TABLE IV. PATIENTS

Name.	*Hours flying.*	*No. of observations.*	*No. of breaths per min.*	*Litres of air per min.*	*Av. vol. of each breath in c.c.*	*Alveolar CO_2 %.*	*Dead space, in c.c.*
Lt. W.	45	4	14·3	8·6	607	5·98	139
Lt. E. M. C. ..	6	4	21·4	9·5	443	5·73	125
Lt. W.	13	5	15·0	6·16	413	5·93	81
Lt. D	100	5	7·5	5·3	707	5·42	—
Lt. C.	160	3	19·5	8·25	423	5·27	110
Lt. A.	1,575	3	24·0	9·8	408	5·76	147
Lt. B.	100	4	21·2	7·8	367	6·01	126
Capt. S.	—	2	21·0	8·4	400	5·76	145
Lt. H.	—	1	19·0	8·2	430	—	—
Capt. H.	600	3	16·0	6·3	393	5·05	90
Lt. B.	100	3	18·5	6·7	262	6·26	88
Lt. R.	—	1	16·0	9·6	600	—	—
Lt. G.	160	3	15·0	7·2	480	5·71	120
Lt. C.	167	2	15·0	8·1	540	5·5	138
Lt. C.	50	2	16·0	6·9	434	5·5	141
Lt. M.	—	1	12·0	6·6	550	5·28	116
Capt. L.	180	3	21·3	8·8	410	5·95	112
Lt. V.	200	3	16·0	9·6	600	5·65	193
Lt. M.	400	2	12·5	5·9	472	5·83	94
Lt. W.	300	4	29·4	8·4	285	5·67	83
Lt. B.	13	2	18·5	9·6	520	5·18	129
Lt. B.	27	2	15·5	7·0	450	5·39	100
Lt. C.	18	1	22·0	9·1	416	5·27	110
Capt. E.	400	1	16·6	9·3	560	5·72	165
Capt. McE. ..	255	1	26·6	8·4	316	5·25	93
Capt. B	440	1	17·2	7·5	439	5·59	94
Capt. C.	250	1	22·0	6·3	280	5·21	48
Lt. H.	60	1	35·0	9·1	262	—	—
Lt. L.	100	1	17·7	7·0	400	—	—
Lt. C.	400	1	28·3	8·2	290	—	—
Maj. M.	1,000	1	19·0	7·3	386	—	—
Lt. D.	500	1	7·0	6·26	890	—	—
Lt. R.	200	1	27·0	8·3	310	—	—
Lt. M.	55	1	12·0	7·58	630	—	—
Lt. C.	30	1	11·0	6·6	600	—	—
Lt. N.	360	1	14·0	5·7	410	—	—
Lt. G.	73	1	23·2	6·8	296	—	—
Average			18·4	7·7	418	5·59	

TABLE V. CONTROLS

Name.	*Hours flying.*	*No. of observations.*	*No. of breaths per min.*	*Litres of air per min.*	*Av. vol. of each breath in c.c.*	*Alveolar CO_2 %.*	*Dead space, in c.c.*
Capt. B.	1,000	1	18·0	8·1	450	5·4	117
St. W.	600	1	18·6	8·3	454	—	—
St. C.	600	1	7·3	9·3	1,272	—	—
Capt. K.	600	1	15·0	9·39	626	—	—
A. H. C.	—	1	22·0	7·8	354	—	—
R. C.	—	1	18·5	7·5	405	—	—
Lt. E.	—	1	14·0	6·1	445	—	—
W. A.	—	1	12·0	7·8	650	—	—
L. G. M.	—	1	11·0	6·8	624	—	—
J. F. P.	—	1	19·0	8·8	463	—	—
A. S.	—	1	17·0	7·8	460	—	—
R. B. D. C. ..	—	1	19·0	8·8	466	—	—
J. S.	—	1	18·0	7·6	422	—	—
Surg. H	10	4	11·5	7·0	610	5·6	132
Surg. S.	—	1	11·0	7·76	705	—	—
Lt. P. J.	—	1	12·3	7·58	616	—	—
Average			15·2	7·9	563		

The Depth of Respiration.

The average depth of respiration is obtained by dividing the volume of air breathed per minute by the number of respirations per minute.

The average figure for 37 patients is 418 c.c. and for the 16 controls 563 c.c. It will be noted that the number of quick shallow breathers among the patients is high ; in ten out of 37, the average depth of respiration falls below 400 c.c., while in the controls, only one out of 16 falls below 400 c.c.

There is no obvious relationship between the depth and rate of respiration and the rate of oxygen consumption. The shallow breathers are distributed fairly evenly between the slighter and the more severe cases.

The Changes produced in Normal Respiration by Breathing through Valves.

It is obvious that a measurement of the depth and rate of respiration obtained by breathing through a mask and valves may not give a true index of normal quiet breathing.

After the series of measurements recorded in Tables IV and V had been completed the use of an enclosed spirometer[1] became available. By means of this instrument a graphic record is obtained of the respiration rate and the volume of air breathed.

A further group of 28 patients, very similar in type to the first group, was examined in the following manner. A record of normal quiet breathing was taken while the subject read a newspaper and paid no attention to his breathing. Then the mask from the Douglas bag apparatus was fitted over the face and the subject was asked to continue reading paying as little attention as possible to the respiration. The results obtained were somewhat varied, and are given in Table VI.

Breathing through the mask produced an average increase of depth of about 5 per cent., at the same time the average rate of respiration was slightly diminished. Individual variations were considerable. Considering the alterations in the respiratory rate first : in 6 cases the rate remained the same, in 7 cases the rate was quickened, in 15 cases the rate was slowed. Extreme variations are seen in case No. 8, where the rate slowed to 14 per min. when the mask was used, as against a normal rate of 22 per min., and in cases Nos. 25 and 26, where the rates quickened considerably with the mask, from 27 to 34 per min. in No. 25, from 16 to 23 per min. in No. 26.

Considering the alterations in the depth of respiration : in 5 cases the volume per breath remained unaltered, in 16 cases the breathing deepened, in 7 cases it became more shallow. Usually the volume varied by about 40 or 80 c.c., in case No. 8 the volume of each breath increased by 120 c.c. with the mask, and in case No. 21, the volume decreased by 120 c.c. (In the graphic record an excursion of 1 mm. represented a change of volume of 40 c.c.)

[1] Provided by the Air Inventions Committee to the design of Sir Horace Darwin, K.B.E.

TABLE VI

No.	Name.	Normal quiet breathing. Rate of Respiration per min.	Normal quiet breathing. Depth of Respiration c.c.	Breathing through Mask and Valves. Rate of Respiration per min.	Breathing through Mask and Valves. Depth of Respiration c.c.
1.	Lt. P.	16	440	15	480
2.	Lt. H.	18	640	16	560
3.	Capt. M.	14	480	12	560
4.	Lt. Y.	16	440	14	520
5.	Lt. G.	16	520	16	520
6.	Lt. B.	18	440	17	480
7.	Lt. A.	19	400	19	400
8.	Lt. E.	22	440	14	560
9.	Lt. K.	14	440	14	480
10.	Lt. L.	18	480	18	440
11.	Capt. C. K.	21	360	17	440
12.	Lt. E.	18	480	20	480
13.	Lt. W...	14	560	18	520
14.	Lt. M.	16	360	14	400
15.	Lt. M.	14	440	16	480
16.	Sgt. McR.	20	360	20	360
17.	Lt. G.	22	300	24	320
18.	Lt. B.	27	440	26	470
19.	Capt. N.	18	420	16	420
20.	Capt. S.	21	320	23	380
21.	Lt. F.	17	480	14	600
22.	Lt. B.	20	360	18	440
23.	Lt. B.	25	320	23	400
24.	Capt. L.	13	600	13	520
25.	Lt. W...	27	300	34	240
26.	Lt. C.	16	400	23	320
27.	Cdt. M.	16	480	14	440
28.	Lt. A.	16	400	15	480
	Average	18·2	432	17·9	454

Three examples of the graphic records obtained are appended. In these tracings the down stroke represents inspiration and the up stroke expiration.

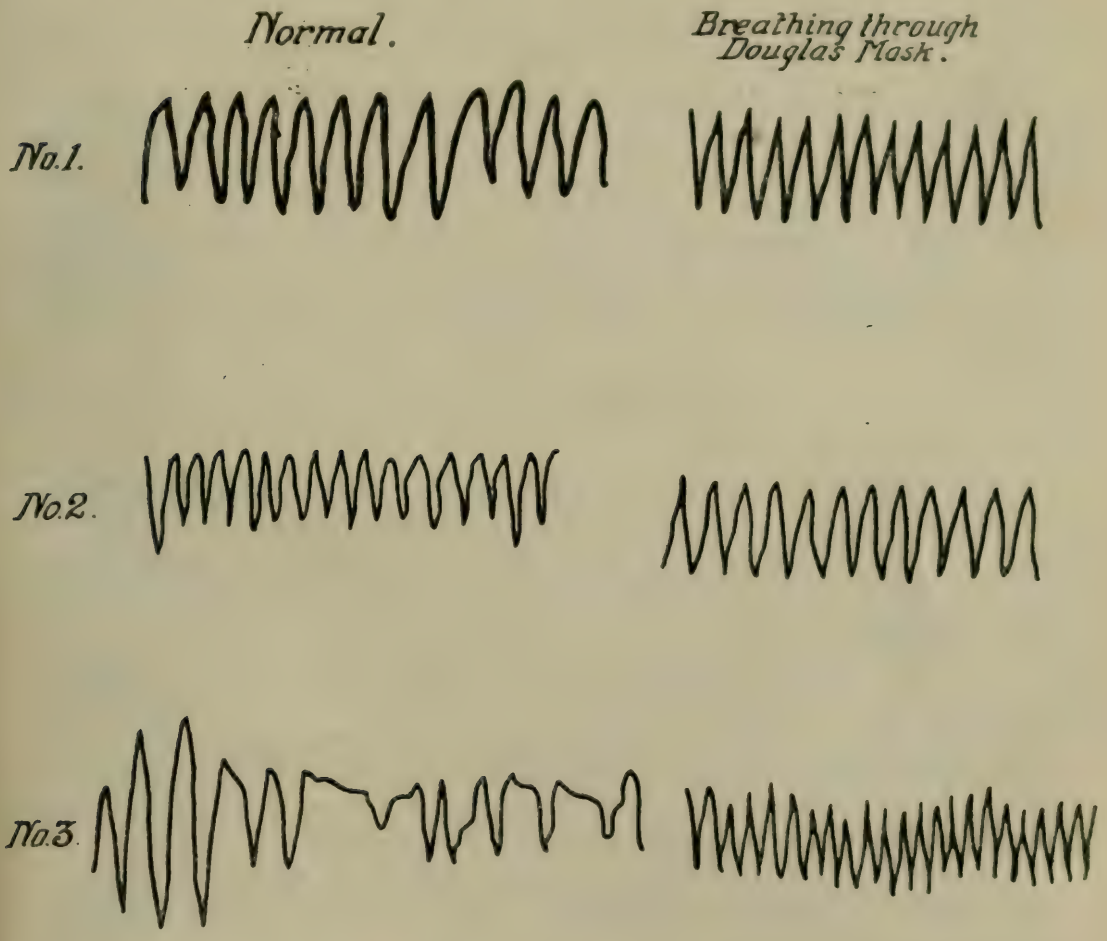
No. 1 is the tracing obtained from a fairly fit man ; the slight expiratory pause is abolished when breathing through the mask. The deepening and slowing of respiration produced by the mask is illustrated in No. 2 tracing.

Patients with marked nervous symptoms usually give a very irregular tracing even when breathing quietly. In most of these cases breathing through the mask produced a more regular rhythm. An extreme example of this kind is seen in tracing No. 3. Here very irregular breathing at a moderately slow rate changes to a rapid regular rhythm. This patient is not included in Table VI, as the tracing of normal breathing is too irregular to measure satisfactorily.

Voluntary Variations in the Rate and Depth of Respiration.

It has been pointed out by Haldane and Priestley that the rate of breathing can be varied within wide limits, provided that the depth of breathing regulates itself, without any forcing or holding the breath. A normal man can breathe comfortably at 3 or 4 a minute or at 60 a minute if the depth of respiration is varied at

the same time. In the course of an investigation on the respiratory dead space at different depths of breathing, patients were asked to breathe as slowly as possible, without holding the breath or without making themselves feel uncomfortable. It was found that normal men and men suffering from a moderate degree of fatigue were able to breathe at 3 or 4 a minute without any discomfort. A certain number of patients seemed unable to regulate at will the rate of breathing. This failure occurred in patients in whom symptoms of a neurasthenic nature predominated. The difficulty appeared to lie in the regulation of the rate, rather than in the regulation of the depth of respiration. For instance, one patient who could only reduce the rate to 9 a minute was able to breathe at a depth of 1,700 c.c.,



a depth which would suffice a normal man breathing at the rate of 3 or 4 a minute.

C. The Alveolar Air.

In a number of cases the carbon dioxide in the alveolar air at rest was determined by the Haldane-Priestley method. Two samples were taken, one at the end of inspiration, the other at the end of expiration. The figures given in Table IV, column 6, represent in each case the mean of two such samples. Where more than one observation has been made, the average for each patient is given.

54 observations were made on 25 cases. The average figure was

5·59 per cent., the minimum was 4·8 per cent., and the maximum 6·63 per cent.

These figures may be compared with those given by Haldane and Fitzgerald :

	Mean.	*Minimum.*	*Maximum.*
Observations on 27 men	5·59%	4·72%	6·34%

Also with those given by Cook and Pembrey :

50 observations on 10 men :	5·57%	4·87%	6·11%

In this series of patients there is obviously no deviation from the normal, as far as the alveolar CO_2, when at rest, is concerned.

D. The Respiratory Dead Space.

The respiratory dead space at rest was determined by the method of Haldane and Douglas. The data required are : the average depth of an expiration (E) ; the percentage of CO_2 in the expired air (e) by analysis of a sample of mixed air in the Douglas bag ; the percentage of CO_2 in the alveolar air (a) ; the dead space of the apparatus (d), i. e. the space between the valves and the face in the mask. The virtual dead space (D) is then calculated from these data by the formula :

$$D = E - E \times \frac{e}{a} - d.$$

The volume of the dead space in a normal man at rest, with an average depth of respiration, is usually given as lying between 128 c.c. and 158 c.c. Haldane has pointed out that the dead space varies with the depth of breathing, and that it may be increased by deepening the respiration.

The dead space, during quiet respiration at rest, was calculated in a number of patients and the figures are given in the last column of Table IV. Average figures are given where more than one observation has been made. It will be noted that while the majority of the figures fall within normal lines, some are on the low side, and on the whole the low figures are found in the shallow breathers.

In a few cases the dead space was calculated when the depth of breathing was varied voluntarily. The following figures illustrate the results obtained.

	Rate per min.	*Depth in c.c.*	*Dead Space in c.c.*
Natural breathing at rest	11·5	534	128
	13·5	511	136
Restricted breathing	30	293	80
Deep breathing	3	1,875	323

Summary.

In a series of pilots and observers, temporarily or permanently unfit for flying, the rate of metabolism at rest as indicated by the oxygen consumption has been found to be slightly but definitely

higher than in a series of fit men. In patients with marked nervous symptoms the rate was usually high.

Prolonged flying, *per se*, does not cause an increased rate of oxygen usage.

The frequent history of concussion in patients with a high rate of metabolism is noted.

The proportion of quick shallow breathers among the patients in this series was unduly high.

There is no evidence to show if the quickened shallow respiration was the result of flying stress, or whether it was present before the strain of flying began.

A restricted dead space was usually found in the shallow breathers.

Determination of the alveolar CO_2 showed no departure from the normal.

11.—THE SENSE OF STABILITY AND BALANCE IN THE AIR

BY

HENRY HEAD, M.D., F.R.S.

(*First published, May, 1919.*)

CONTENTS

PAGE

A. INTRODUCTION 215

B. THE TESTS AND WHAT THEY REVEAL 218

(*a*) Tremor 218
(*b*) The Knee-jerk 219
(*c*) Walking a line heel to toe and turning on one foot . . . 219
(*d*) Standing on one foot for 15 seconds with the eyes closed . . 220
(*e*) Balancing a rod on a flat board with the eyes open or closed . . 222
(*f*) Conclusions 223

C. 'GIDDINESS', VOMITING, AND 'FAINTING' IN THE AIR . . . 224

(*a*) Introduction 224
(*b*) 'Giddiness' associated with temporary Abnormalities of the Auditory Apparatus 226
(*c*) 'Giddiness' in the Air with no abnormal Conditions in the Auditory Apparatus 228
(*d*) 'Fainting' in the Air 235
(*e*) Comparison of the two reactions, 'Giddiness' and 'Fainting' in the Air 237
(*f*) Attacks of minor Epilepsy in the Air 240
(*g*) Conclusions 241

D. SENSATIONS EXPERIENCED BY NORMAL PERSONS IN MACHINES HEAVIER THAN AIR 243

(*a*) Introduction 243
(*b*) Actual Experience during Aerobatic Evolutions . . . 245
(*c*) The Human Element in Aeroplane Accidents:
(i) The Physiological Effects of disturbances of Equilibrium . 250
(ii) Regression 252

E. GENERAL SUMMARY 254

A. Introduction.

The faculty in man, which has raised him to a dominant position in the animal kingdom, is his capacity to make use of tools. He projects his powers of appreciation to the extremity of some mechanical contrivance, manœuvred by hand or foot, and so enormously extends his power to influence objects at a distance from his body. When we probe a wound or use a stick to explore the ground, we 'feel' at the end of the rod, not in our fingers. From such comparatively simple acts we can rise to the most complicated adjustments of eye and hand. For example, when shooting, our eyes are fixed on the bird passing across the field of vision; the gun is then thrown up with a movement so exactly regulated in time and direction, that the shot will arrive at a certain spot at the precise moment when the flight of the bird brings it to that position in space. We do not 'aim' or calculate some hypothetical distance 'in front'; nor do we actually see the gun at all. We carry out a reaction exactly adapted to the information given by our eyes. The more automatic this response the more likely is it to succeed in bringing down the bird.

Man not only uses this power of projecting himself to the end of some mechanical appliance in his work, but games were invented to exercise this faculty. Flying a machine heavier than air is nothing more than an extended application of this power, demanding adapted movements of every part of the body and limbs. No new elements come into the mechanical problems of flying that are not evident in riding a motor-bicycle, game shooting, cricket, or golf.

The vast majority of young men can acquire any of these aptitudes. A small minority are so constituted that they cannot do so; they 'do not feel safe' on a bicycle, and 'cannot hit the ball'. Such persons are rare amongst the healthy members of the community; but they form a small group who are 'not fond of games'. At the other end of the scale stand a small number of super-men, who seem to have been born with a natural gift, which rapidly carries them to a position above the average of their fellows.

Amongst candidates for the R.A.F. our aim must be to exclude those who are congenitally incapable of flying. At the same time we cannot hope to pick out super-men. We are concerned with the average youth who is capable of adjusting himself to conditions in the air. Any healthy young man, who is able to ride a motor-bicycle and play cricket or lawn tennis well, can be taught to fly.

Were this the whole problem it would not be difficult to select suitable candidates. But it must not be forgotten that the R.A.F. is a fighting force. A man must not only be a good

chauffeur, but he must be able to fight. I examined a pilot, who was acknowledged to be a remarkable aviator capable of flying any machine and apparently fearless in the air. But he was sent back from France because he could not fly over the lines. On the other hand, I have come across pilots with a fine fighting record, who were not good flyers in the technical sense, and neither knew nor cared anything about the details of their machine.

At first, candidates for the flying branches of the two services were admitted after a medical examination which varied greatly in strictness. But, with the coming of the Military Service Act, the rush of volunteers greatly increased. This enabled the authorities to raise the standard of the medical examination; but at the same time its aim was somewhat extended. An attempt was made, not only to exclude those who showed signs of pathological states, organic or functional, but to select men who would be able to fly well.

The various functions which come into play during aviation were analysed, and methods were devised to test the physiological activities on which they were supposed to depend.[1] At the same time the importance of the abnormal states induced by flying at high altitudes led to the invention of simple cardio-vascular and respiratory tests, which were found to be of value in appraising the general medical condition of a candidate or flying officer.

In its final form the medical examination of the Candidates Board was intended to fulfil a threefold object:

(1) To exclude all who suffered from pathological conditions. These included, not only gross defects of the heart, lungs and nervous system, but also such functional defects as 'nervous instability'.

(2) Some persons of normal constitution are liable to suffer in the air from certain grave disabilities, such as 'giddiness' and 'fainting'. These states are no more evidence of a pathological condition than is sea-sickness or inability to swing without discomfort. It was hoped that by applying tests which made a call on the activity of the semicircular canals, muscular sensibility and other fundamental sensory functions, it would be possible to exclude candidates who would suffer from these disabilities in the air.

(3) A certain proportion of pupils are rejected because they have difficulty in learning to fly; they are 'heavy on controls', 'make bad landings', or 'have no air feeling'. It was hoped that tests, founded on the sensations underlying balance, would exclude candidates who might show these defects.

On the protocol (Form C.B. 7) of the R.A.F. Commissions Board are three headings, 'Nervous Stability', 'Muscle Sense', and 'Vestibular Stability'; it is with these we are mainly concerned in the first part of this report.

[1] All the tests for balance were invented and carried out by Arthur H. Cheatle, F.R.C.S., who directed the Otological work of the Candidates Board.

In actual practice his opinion was based on the following methods of examination:

(1) The '*Nervous Stability*' of a candidate was judged from his past history, revealed by a few direct questions; by the evidence of nail-biting, tics or other signs of want of control, such as tremor of the hands and inability to balance a rod on a flat board with the eyes open.

(2) The condition of the '*Muscle Sense*' was estimated by the manner in which he balanced a rod on a flat board with his eyes closed and by his capacity to stand on one foot for 15 seconds under the same conditions.

(3) As tests of '*Vestibular Stability*' the candidate was asked to walk along a line heel to toe and to turn completely on one foot, so as to face in the opposite direction. The character of the entry under this heading was also determined by the manner in which he stood on one foot with his eyes closed.

Thus it is obvious that three mechanical tests were habitually relied on to give the information required. First, the candidate was made to extend his arms and hands to see if he was steady or tremulous. Then he was made to stand on one foot for 15 seconds and then on the other, both with the eyes open and shut; he was also made to walk along a line and turn at the word of command. Thirdly, he was made to balance a rod on a flat board by means of each hand in turn, with his eyes open and shut.

But, before any conclusion can be reached concerning the correlation of these tests with ability to fly, the results must be recorded in terms of the actual methods employed. No general opinion should be given such as 'Muscular Sense defective'; the actual facts of the examination must be recorded.

Unsteadiness, when standing on one foot with the eyes closed, was supposed to represent want of 'Muscle Sense'. I therefore examined a number of pilots and pupils, who were unable to carry out this test on either foot, for their power of recognizing passive movement at the various joints of the lower extremities. Now we know from other lines of research that this is the most delicate of all the measurable tests for what is usually called 'Muscle Sense' or 'Deep Sensibility'. So fine is the power of perceiving passive movement at all the joints from the great toe to the hip, that the normal individual can appreciate without difficulty a range of 1° or even less. This is scarcely perceptible to the eye and yet its direction can be accurately described by any intelligent person. Amongst the pilots and pupils who were unable to stand on one foot with the eyes closed, I did not find a single one who could not appreciate 1° of passive movement at all the joints of the lower extremity. It is obvious, therefore, that in the class of young men with whom we have to deal, inability to carry out this test has nothing to do with defective 'Muscle Sense', but is due to lack of motor control or want of resolution.

The condition of 'Muscle Sense' in the upper extremities is supposed to be revealed by the manner in which the rod is

balanced on a board, when the eyes are closed. I have carefully measured the power of recognizing passive movement in a series of pilots and pupils who were unable to carry out this test; records from the joints of fingers, wrist, elbow and shoulder, showed that they had no difficulty in appreciating a movement of 1°. Evidently, in the class of case from which our material is drawn, failure to carry out this test has nothing to do with defective 'Muscle Sense'.

'Vestibular Stability' is estimated by the power to walk along a line, to turn on one foot and to stand on one leg with the eyes closed. But there is no evidence to show that any of these acts bears a direct relation to the activity of the semicircular canals. Moreover, I have examined four pilots who were intensely giddy and vomited in the air, none of whom had the least difficulty in carrying out these tests perfectly.

B. The Tests and what they Reveal.

Although success or failure in these tests does not reveal the state of the 'Muscle Sense' or extent of 'Vestibular Stability', they form a valuable guide to the patient's physical and mental condition. I shall therefore consider these different methods of examination one by one, pointing out under what circumstances they are capable of yielding useful information. The material at my disposal consisted of 30 cadets, in 22 of whom I was able to obtain the after history; 20 pupils who were rejected for some inability to fly of a non-medical nature; 30 cases where a pilot or observer came to me on account of some disability such as vomiting or fainting in the air. In addition I have gained much useful experience from the patients under my care who passed through the annexe to the R.A.F. Central Hospital (Oakhill House).

(*a*) *Tremor.*

When a young man is made to hold his hands out in front o him, and at the same time to protrude his tongue, any considerable tremor is a sign of some definitely abnormal condition. A tremulous tongue may be indicative of chronic indigestion or of alcoholic excess; unsteadiness of the fingers, especially when they are abducted and brought together again, is a certain sign of lack of control. Tremor is not so much an indication of a neuropathic temperament as of a definite disturbance of function. It is not surprising therefore that Major Bowdler concluded that the group which contains 'tremor' showed a high correlation with want of aptitude to fly. He found that out of 54 candidates who were accepted in spite of 'marked tremor', 14 did not qualify, a ratio of success to failure of 2·8 to 1. Amongst 2,000 candidates who showed no abnormal signs 7·6 per cent. only were ultimately rejected.

The number of completely normal young men who show

tremor of the hands must be extremely small. In every case of a pupil, pilot, or observer that has come under my notice, this sign has been associated with some obvious cause. In a certain number it was due to alcoholic excess, and it was particularly evident in those pilots who took alcohol in order to be able to carry on their work. But, apart from alcohol, it is one of the commonest signs of stress of war service and was particularly frequent in those candidates who had already served in the infantry at the front. It was almost universally present in those officers who were admitted to hospital for some functional psychosis ('Shell Shock').

Tremor of the hands is a not infrequent sequel to malarial infection; it is more evident in delicate and precise movements, such as shaving, and the handwriting may be affected. Such unsteadiness comes out particularly well when attempting to balance the rod on a flat board.

Excessive smoking, especially the perpetual misuse of cigarettes, is responsible for much tremor of the hands in members of the R.A.F.

Thus, tremor is evidence of some disordered functional state, and should be looked for definitely in the course of the routine medical examination of candidates or pilots. It will be found most commonly amongst those who have been sent back from the front on account of stress of service, exhaustion, or states of anxiety.

(*b*) *The Knee-jerk.*

The condition of the knee-jerk is of little importance in the class from which the candidates for the R.A.F. are drawn. They are all young. None of them have reached the age at which syphilis is likely to have attacked the mechanism of this reflex, and 'exaggerated' knee-jerks are of little significance. Major Bowdler could find no evidence that any flying disability was associated with 'exaggerated reflexes'.

A condition not infrequently called 'exaggerated knee-jerks' is, however, an indication of loss of general control, but has in reality nothing to do with tendon reflexes. Sometimes on tapping the knee the whole body is thrown into agitation and the patient may even cry out. If the knee is carefully watched, the true knee-jerk is seen to precede the general commotion. This 'exaggerated knee-jerk' is in reality an uncontrolled reaction to a gentle blow and the same person will be found to start at a sudden noise. This 'general response' to tapping the knee is a valuable sign of loss of control, but is no indication of the condition of the deep reflexes. It is usually associated with a state of anxiety and is more likely to be found in unfit pilots than in candidates for the R.A.F.

(*c*) *Walking a line heel to toe and turning on one foot.*

The result of this test is recorded by the Examining Board under the heading of 'Vestibular Stability'. But there is nothing

to show that inability to walk a line and to turn on one foot is evidence of anything more than clumsiness in action or slowness of comprehension.

We examined a number of cadets, all of whom had been passed by the Candidates Board and had already begun to fly. We were able to obtain the ultimate history in 22 cases; 13 flew well and amongst them 9 could walk a line and turn on one foot well, but 4 carried out this test badly. Amongst the 9 pupils who failed to qualify, 3 walked and turned well, whilst 6 were bad at this test.

Such numbers are useless for any statistical comparison; but certain points came out on closer personal examination of those who failed. One pupil showed astonishing lack of comprehension of the task he was asked to perform; when told to turn on one foot only, he walked round in a circle and repeatedly turned in this way, although we demonstrated to him the manner in which we wished him to carry out the test. This man did 6 hours and 25 minutes dual flying; but when the time came to go up alone, he 'went sick' and did not fly again.

The same failure to comprehend what was required was apparent to a less degree in two other cases amongst the pupils who were finally rejected. It has nothing to do with want of stability, but is evidence of a slow response to unexpected verbal commands.

Another cadet, who had fought successfully in France, found considerable difficulty in walking heel to toe and turning on one foot. He ultimately became an excellent scout pilot, but confessed that he disliked dancing. In fact it seems to us that this test is more likely to show ability to dance than any capacity to fly.

But whatever relation it may bear to flying it is certainly not evidence of 'Vestibular Stability'. For, out of 8 pilots who suffered from profound giddiness associated with vomiting in the air, two only failed to pass this test; both of these men were tremulous and showed other neuropathic signs and symptoms.

(*d*) *Standing on one foot for* 15 *seconds with the eyes closed.*

If a candidate is unable to carry out this test, his failure has been attributed to defective 'Muscle Sense'. But I carefully tested the power of recognizing passive movements at the various joints of the lower extremities in several R.A.F. pilots and pupils, who failed to stand on one foot with the eyes closed. I selected the grossest cases only; but in no single instance was the power of recognizing posture and passive movement affected. Moreover, these same persons, who were unable to stand on one foot, could do so with both feet on the ground even when the eyes were closed; Romberg's sign was completely absent, showing that the impulses passing up the posterior columns of the spinal cord were not affected. There is no evidence, therefore, that inability to stand on one foot for 15 seconds with the eyes closed

bears any relation to disturbed sensations from the muscles and joints, in the class of young men with whom we have to deal. Moreover, all the pilots and pupils who failed to carry out this test in spite of repeated attempts, showed considerable tremor of the hands and were evidently suffering from some neuropathic or psychopathic condition.

Amongst the cadets who ultimately proved to be successful pilots, two carried out this test poorly. Both showed some tremor of the hands, and one of them had come home after fighting in France to join the R.A.F. Of the nine unsuccessful cadets, three showed defective power of standing on one foot, and of these two had distinct tremor of the hands.

Amongst 20 healthy pilots who were rejected on account of inability to fly and not for any physical reason, 15 carried out this test perfectly. Of the five who could not stand on one foot for 15 seconds on the first attempt, three were able to do so on a second trial and two only failed three times on each foot.

But amongst 20 pilots who came under my care for some pathological condition, such as vomiting or fainting in the air, fatigue, or anxiety, 11 showed gross defects in carrying out this test. Thus if we place together in one group the unselected cadets and the officers who had been rejected for defective flying capacity, we obtain 42 healthy individuals; amongst them were 10 cases where this test could not be carried out perfectly. On the other hand, of 24 pilots and pupils who ceased to fly because of some morbid condition in the air, 15 showed considerable difficulty in balancing themselves on one foot.

Thus it is evident that amongst candidates, pupils, and pilots of the R.A.F., inability to stand on one foot with the eyes closed for 15 seconds is evidence of some want of control and is not due to defective 'Muscle Sense'.

Secondly, this test is more likely to be badly executed by those who show other signs and symptoms of fatigue or anxiety, whether temperamental or acquired. It is not directly a test for flying capacity; but a man who has difficulty in carrying it out successfully will probably be found to be suffering from mental or physical stress. This will ultimately affect his handling of machines heavier than air, although it may have no influence on his work as an airship pilot.

To stand on one foot with the eyes closed for 15 seconds requires not only perfect automatic control but some determination. One of the respiratory tests invented by Colonel Flack also calls for a certain amount of resolute effort, and it is interesting to notice the frequency with which the results of the two methods fall together. The pilot under examination is told to blow into a U-tube and to support a column of mercury 40 mm. in height for as long as possible. Forty seconds is taken to be normal, although a healthy young man usually greatly exceeds this period. But the result is gauged not only by the time but also by the effect on the patient; if he becomes suffused and distressed he is considered to have failed even if he slightly exceeds 40 seconds.

Amongst 20 pilots who suffered from some abnormal condition in the air, 9 failed to pass both the balancing and U-tube tests. One showed defective power of standing on one foot, but supported the mercury for 45 seconds; one other failed in the respiratory test, but succeeded in balancing himself on one foot.

This association between the results of the two methods of examination might have been anticipated. Both depend on sustained effort, and anything which diminishes the power of concentration will influence both tests. All the 9 pilots who failed were suffering from fatigue, anxiety, want of sleep, bad dreams, or other analogous symptoms; it is such states which above all others are associated with defective concentration and diminished control.

(*e*) *Balancing a rod on a flat board with the eyes open or closed.*

This is an easy test to carry out and requires no apparatus that cannot be improvised out of the lid of a cigar-box and a small tuning-fork with a flat base. The board is placed on the table with the rod standing upright on that part further from the person to be examined. He is then asked to grasp the edge nearest to him with his fingers, to raise the board to the level of his shoulders and to replace it on the table without upsetting the rod. Should he fail at the first attempt with either hand, he is allowed to try three times in succession. He is then examined in the same way with the eyes closed.

Failure to balance the rod with the eyes open was assumed by the Examining Board to be evidence of want of 'Nervous Stability', a heading shared with Tremor, Tics, and other symptoms of want of control. But inability to pass this test with the eyes closed was classed as due to defective 'Muscle Sense'.

I therefore selected for closer investigation several of the worst cases, where the subject of examination failed entirely to balance the rod with either hand, when his eyes were closed. In no instance could I find any measurable defect in 'Muscle Sense'; 1° of passive movement could be accurately appreciated at all the joints of the upper extremity. It is evident, therefore, that, amongst pilots and candidates of the R.A.F., failure to balance the rod with the eyes closed is not dependent on lack of Muscular Sensibility. It is, in fact, simply another and more severe method of examining the same want of control and clumsiness of action, betrayed by balancing the rod with the eyes open.

Most normal persons find greater difficulty in balancing a rod on a flat board with the eyes closed than when they are open. Now, whenever some function of the nervous system is defective, the more complex and difficult task shows evidence of failure before one that is simpler and easier of execution. It is not surprising, therefore, to find that out of 20 cadets who had already begun flying, 4 showed some difficulty in balancing the rod with the eyes open and 8 with the eyes closed; but this

difficulty bore no relation to their subsequent career as aviators. Moreover, amongst 20 pupils rejected solely because of inability to fly, 5 had some difficulty in balancing the rod with the eyes open, 10 with the eyes closed. Thus if we compare the normal cadets and rejected pupils there is nothing to show that there was any material difference in behaviour between those who could and those who could not fly, provided they were physically healthy.

But when we turn to the 25 pilots or pupils who suffered from abnormal symptoms in the air, the proportion of failures was nearly double as great; 13 had difficulty in balancing the rod with the eyes open, 19 with the eyes closed. Evidently this test reveals some condition common amongst those who have suffered from pathological states in the air. But provided the individual is otherwise healthy, it does not bear any direct relation to flying capacity.

On looking through the records of those who failed more or less grossly in this test, the high proportion of cases of tremor is at once evident. This might have been anticipated; for any tremor makes it difficult to carry out an operation which requires a steady hand. Out of 28 pilots who had suffered from pathological states in the air, 14 were tremulous and all but one showed difficulty in balancing the rod on the board. In the same way, out of 50 cadets and rejected pupils 11 showed some tremor of the hands, and all of these failed to balance the rod satisfactorily.

But it must not be supposed that this test is only another means of discovering tremor. Amongst 28 pathological cases there were 7 persons who were not tremulous but could not balance the rod with normal certainty. In the same way, amongst 30 cadets and 20 rejected pupils, 9 could not carry out this test satisfactorily, though free from tremor. In almost every instance of this class I made a special note that the failure was due to erratic conduct, carelessness, want of resolution, or defective comprehension of the task and the best method of carrying it out. In other cases the movements were 'clumsy' and 'stiff', or the subject seemed 'utterly worn out' and 'tired'.

Thus we may conclude that, provided the subject of examination is in perfect physical health, this test has no direct bearing on his capacity to fly a machine heavier than air. But, on the other hand, it is a useful method of detecting states of exhaustion, flying-stress, insomnia, and other neuropathic and psychopathic conditions, in an early stage of development. There is no difference in principle between the test carried out with the eyes open or shut. The latter is a more difficult task, and therefore tends in most cases to show defects sooner than when the balance is guided by sight.

(*f*) *Conclusions.*

Some of the methods of examination discussed in this chapter are of sufficient value to form part of the routine medical

examination of candidates and pilots of the R.A.F. Tremor of the hands and tongue must always be carefully noted. The candidate should be asked to stand first on one leg and then on the other for 15 seconds with the eyes closed. He is also made to balance a rod or tuning-fork on a flat board with either hand in turn, first with the eyes open and then with them closed. He is allowed three attempts, and the character of his failure to carry out this test should be recorded, if possible.

In every case the actual method of examination must be recorded, and all such general categories as 'Muscle Sense', 'Vestibular Stability', and the like, must be avoided.

With such material as we meet with in the R.A.F., consisting as it does of young men in whom gross organic disease is almost absent, these tests reveal the condition of physiological or psychical control. Such control may be defective from innumerable causes; some of the most potent are fatigue, physical or mental stress, anxiety and fear. All of these conditions may appear in the course of the work of any aviator, but were enormously aggravated by flying under war conditions.

C. 'Giddiness', Vomiting, and 'Fainting' in the Air.

(*a*) *Introduction.*

A pilot may lose control and become unconscious in the air from many various causes. Of these want of oxygen at high altitudes is perhaps the best known, and has formed the subject of several previous Reports. It is not my intention to deal with this condition, but to consider abnormal symptoms which may occur at any height and are not due to physical or chemical deficiencies in the atmosphere.

These are usually called by the sufferer 'giddiness', 'dizziness', and 'fainting in the air'. Unfortunately many medical men record them as 'vertigo' and 'syncope', without any qualification or explanatory statement of what actually happened. Nothing could be more fatal for the progress of knowledge than this widespread tendency to translate popular nomenclature into nosological language. To say that an aviator suffered from 'vertigo' inevitably suggests that the cause of his trouble was the semicircular canals, whilst 'syncope' attracts attention to the cardio-vascular system.

Throughout this chapter I shall allude repeatedly to the way in which the patient reacts when rotated in a chair. American workers have laid great stress on this response to rotation, and have carried out their observations in such a way that each group of semicircular canals is stimulated in turn. In fact, this method of examination formed, at one time, the determining test for admission to their Flying Service. A candidate was

obliged to react in a certain manner or he was not accepted. The Report of the American Surgeons states—

'It was decided to reject applicants whose vestibular apparatus gave evidence of motion-sensing acuity below a certain degree, albeit it was fully recognized in establishing this limit, that it in no way represented a line of demarkation between acuities of this perception compatible with and incompatible with flying.'

The extent of the response of the vestibular apparatus to disturbances of equilibrium was estimated in two ways. First, the candidate was rotated with his head in a strictly regulated position and the duration of the nystagmus was measured. Secondly, without rising from the chair and with his eyes still closed, he attempted to find the observer's finger, which was placed in a certain, previously familiar, position. If the deviation to this test did not reach a certain amount the candidate was assumed to be endowed with less than the normal vestibular acuity; if, on the other hand, it exceeded certain limits, arbitrarily fixed, he was rejected as over-sensitive. But this method of examination is purely empirical, and the data obtained have not so far been correlated with ability to fly.

I have not attempted to check these results; for throughout this work I have used the rotating chair, not as a method of measuring the response of the semicircular canals, but as a means of inducing an abnormal reaction. A healthy man responds in a certain way to rotation, although the degree of giddiness varies greatly. But in certain persons, who suffer from morbid phenomena in the air, the reaction is abnormal and excessive. It is frequently possible, by rotation, to induce physiological states and sensations analogous to those experienced in the air; the disability can be reproduced in a minor form. This is true, not only in cases of 'giddiness', but also in those where the pilot has 'fainted' in the air.

When we sit upright in a chair, the horizontal canals are not strictly in a plane parallel to the ground; they are slightly tilted and the head must be inclined to about 30° before they become strictly horizontal. But this is not the natural position; the head is usually carried so that the eyes look directly forwards and not on to the ground. This is the posture to which we are adapted in daily life, and the head can be turned rapidly to the right or left without giddiness or abnormal symptoms; for these movements occur more frequently than any others under natural conditions. And yet at no time, either during this static pose or on lateral deviation, are any of the semicircular canals in a position suitable for exclusive physiological stimulation. If we want to test the response of the horizontal canals specifically, the head must be suitably adjusted before rotation; but if, as is my intention, we wish to study the general functional reaction of the patient, he must be placed in the natural position seated upright in the chair.

It is important to understand the different aims of the two methods of carrying out this test. Those who carefully place the head in such a posture, that each set of canals is stimulated in

turn, look to the specific nature of the physiological response. On the other hand, I was anxious to determine, not the afferent results of such stimuli, but the way in which the patient could adapt himself to somewhat violent sensory impressions. My object was to examine his control in face of disturbances of equilibrium. Similarly, I not infrequently tested his reaction to a slight blow on the knee or to a sudden noise.

When a healthy man is rotated ten times in twenty seconds with his eyes closed, the results depend mainly on the position of the head. I have not attempted to modify its posture so as to stimulate any one set of semicircular canals alone; but the patient was directed to sit upright in the chair, as if he was gazing directly forwards. On opening his eyes the room seems to be going round in a direction opposed to that in which he was rotated. A normal man is not giddy, and suffers from no nausea, headache, or other discomfort.

If he is asked to rise and walk forwards he may tend to deviate, but rapidly recovers himself and reaches the table, five paces in front of him, without difficulty. When the chin has been held somewhat downwards, so as to bring the one set of canals strictly into the horizontal position, this deviation tends to take place in the direction of the rotation. But with the head in the erect position this is not the case in most instances; thus rotation clockwise is usually followed by more or less deviation to the left.

It is most important to record all rotation, actual or apparent, in terms of the long axis of the body and not as movements to the right or left. But deviation on rising from the chair is described as 'to the right' or 'to the left', because this is the only movement that can be actually observed, although the patient is often aware that it is the expression of a rotatory sensation.

In all my cases the patient was first turned 10 times in 20 seconds clockwise. Then, after a pause, varying in length according to the severity of the reaction, he was rotated in the opposite direction. Usually this second stimulation produced a somewhat greater effect than the first, particularly in subjects who showed other evidence of some abnormal condition.

(*b*) *Giddiness associated with temporary abnormalities of the Auditory Apparatus.*

In order that we may be able to adapt ourselves to changes in position the auditory apparatus must be structurally sound. This is assured in most cases by the careful examination of candidates before they are accepted into the R.A.F.

But Sydney Scott[1] has pointed out that giddiness and other allied symptoms may occur in aviators who are unable to obtain a free and immediate passage of air through the Eustachian

[1] *Journ. of Laryngology, Rhinology, and Otology*, Feb. 1919, Vol. XXXIV, No. 2, p. 51. A fuller account of these researches is also published as a Report of the Air Medical Investigation Committee (Special Series, No. 37).

tubes. The ears may be normal, but the pilot suffers from a cold in the head. The air no longer passes freely through one or both Eustachian tubes and, when he rises to a considerable height or descends with great rapidity, an abnormal condition of tension is produced in one or both middle ears. This, acting on the vestibular apparatus, evokes intense giddiness and is accompanied by pain of more or less severity. Not infrequently effusion, or even haemorrhage, occurs into the middle ear.

Most experienced pilots are conscious of the salutary effect of swallowing during a rapid descent, and habitually chew gum for this purpose. But, in some cases, a temporary condition of the nose or throat may make it impossible to regulate the tension within the middle ear, with disastrous results to the pilot.

In the following instance the giddiness occurred whenever the patient caught a cold in the head, although his auditory apparatus was otherwise normal.

Case No. 1. 2nd Lieut., aged 19.

He first went into the air in September 1917 and had flown for 13 hours solo on Avros before he felt any discomfort. In December 1917 he caught cold, when sleeping in a canvas hangar with snow upon the ground. He went up one morning, with a cold in the head, and suddenly 'felt dizzy'; his head seemed 'to be whizzing round inside', and he 'felt strange'. He went up frequently during the next few days and was 'giddy' on several occasions. He did not vomit and was not nauseated. Throughout this period he was deaf.

He went away on leave, and recovered entirely. On returning to his squadron, he flew solo for 9 hours in perfect comfort, and graduated on April 9, 1918.

He was then transferred to another squadron to fly service machines. Here he caught another cold, and one day, when in the air, whilst suffering from headache and running at the nose, he felt himself becoming giddy at 3,000 feet. He shut off his engine, but does not know how he came down. He was flying an Avro and, according to the report of his Wing Commander, started to spin. He stalled the machine at about 30 feet, and broke some struts in landing, but was not hurt.

He was seen by me in September 1918, four months after the last occasion on which he had been in the air.

He was then a well-built young man with somewhat narrow nostrils. He had been operated on successfully for adenoids at the age of 11 years, and there was no obvious abnormality in his throat or nose. His hearing was good, and the membranes were normal and not retracted; but he said that, whenever he caught a cold, something went wrong with his ears.

Respiratory and cardiac tests showed no gross abnormality, but his balance on one foot was bad. He failed three times in succession to stand on the right foot for 15 seconds, and succeeded with difficulty in doing so on the left. He balanced the rod on the flat board at the first attempt with the right hand, when his eyes were open, and on the second attempt with the left hand. But with the eyes closed, he failed altogether to carry out this test with the left hand, and only succeeded on the second attempt with the right. And yet, on testing his power of appreciating passive movement, he could recognize 1° at all joints of both upper and lower extremities.

When he was rotated, he did not deviate definitely in either direction, and touched the spot on the table perfectly. But he complained that, as soon as he rose from the chair, he felt as if he was 'moving backwards and

forwards'. He seemed to move 'first from right to left, and then back again from left to right'. The window in front of him 'seemed to be doing the same, wavering backwards and forwards'. He was not giddy, and felt perfectly normal, as soon as the 'wavering' passed off. There was no nausea or headache.

From this case there is an easy transition to such a one as the following, where the throat was in an obviously abnormal condition.

Case No. 2. Lieut., aged 27.

He joined the R.F.C. in June 1917, and went to France on January 31, 1918, flying S.E. 5. He began to notice that, when coming in after high scouting, he was a little deaf in the left ear. When he descended from a height, he had to 'blow up his ears', and 'sometimes they would stay plugged for three or four days'.

On April 3, 1918, whilst coming home to the aerodrome, he got into a backwash and went 'dizzy'. He spun from 4,000 feet to 2,500 feet, but did not lose consciousness. He knew what was happening, pulled his machine out of the spin and followed the others home. On alighting from his machine, the giddiness continued for some time.

I saw him nine days later and his tonsils were red and swollen, the left more so than the right. The membranes were normal, and he was not then deaf.

Both these cases are examples of the ill-effects produced by uncorrected changes of tension within the auditory apparatus. Sydney Scott has shown, from careful examination of a large number of pilots at the front, that such abnormal conditions in the middle ear are amongst the commoner causes of giddiness in the air. A healthy man is suddenly exposed to unnatural stimulation of the essential sensory mechanism due to uncorrected tension in the auditory apparatus; he responds in a normal manner with a more or less acute vertigo. Here it is not the reaction of the individual or the response of his semicircular canals that are in fault, but the abnormal and unequal tension in the middle ear.

But 'giddiness' may occur in aviators apart altogether from such external stimuli. In such cases their abnormal state in the air must be due to one or two causes. Either the sensory impressions evoked by changes of bodily posture must be excessive, or the general resistance to the uncontrolled acceptance of such impulses must be profoundly diminished. This defective power of integration and control may be acquired or constitutional. Such cases are of profound diagnostic and scientific importance, and form the subject of the next two sections of this chapter.

(*c*) *Giddiness in the Air with no abnormal conditions in the Auditory Apparatus.*

Most healthy persons can be made giddy, provided the rotatory movement is sufficiently violent, and especially if the head is held in some abnormal position. This reaction is not a patho-

logical condition; it is a normal response to an acute disturbance of equilibrium. But the ease with which it can be evoked varies greatly; some, in childhood, were not able to swing without discomfort and were sick on a train journey. As they grew up, however, this response was evoked less easily, and, provided the stimulus was not unusually severe, they differed little from their fellows.

In the same way many normal men become giddy when first exposed to rapid changes of posture in the air. After a few turns the instructor carries out some aerobatic evolution and, when first looped, rolled, or spun, many pupils become uncomfortable and a certain number vomit. But the majority rapidly recover and may become expert and bold aviators. They have adapted themselves to the new conditions; the stimuli from the peripheral end-organs no longer dominate the field of response. They are controlled or suppressed, not by the will, but by that power of adaptation which is one of the most potent factors in the activity of the central nervous system. In the course of evolution the lower centres have come increasingly under the dominance of those of higher functions and more recent development. Actions, which in lower animals can be carried out by the spinal cord alone, become impossible in man without the co-operation of the centres in the mid-brain.

Even in the fully developed nervous system of man the acquisition of some new aptitude is associated with an increasing control of higher centres. We learn to adapt ourselves to the conditions around us and cease to be the victims of unco-ordinated sensory impressions.

In order that any of these acquired aptitudes may be carried out in a perfect manner attention must be concentrated on the end and object of the movement, and the motor act must become as nearly as possible automatic. A man shoots his best when his eye is fixed on the bird and he is not conscious of his gun. In the same way an aviator must be able to concentrate on the evolution he wishes to perform and carry out the necessary acts automatically. To turn a corner on a bicycle or to bank an aeroplane 'you just lean over' and the desired change occurs without further thought. The man who flies by verbal rules is a bad pilot and will certainly come to grief.

Anything which diminishes the power of concentration tends to lessen control over the reaction of the lower centres to sensory stimulation. An attack of influenza, a 'crash', domestic worry or fear, all act in the same direction. A pilot who was giddy when he first went into the air, but recovered completely, may fall back to his original mode of reaction and be unable to carry out any aerobatic evolution. Such persons are no longer normal, not because the response is a pathological one, but because, for a time at any rate, they have been reduced to a lower level of functional efficiency.

My first example is that of a perfectly healthy man, who never recovered from the tendency to become giddy and to vomit in the air. The case is an instructive one, because he succeeded in concealing his disability and was only saved from death in France

by the prompt assistance of two colleagues. Moreover, since he was marked unfit for flying, he has repeatedly been in the air as a passenger during the course of his work, and appreciates exactly the conditions which render him uncomfortable.

Case No. 3. Capt., aged 23.

After serving in the Infantry he was transferred to the R.F.C. and began to fly on June 15, 1917. He graduated in October and went to France as a Scout pilot on November 14, 1917, flying Sopwith Camels. He did 150 hours in the air, of which 50 were in France.

The first time he was taken into the air on an Avro he vomited at about 2,000 feet. So long as he flew straight he managed without difficulty, but when he turned rapidly or 'threw the machine about' he was attacked with nausea and occasionally vomited. By coming down whenever he was uncomfortable, and by doing as little 'stunting' as possible, he managed to conceal his disability and was sent out to France after 80 hours in the air.

Here he felt no discomfort at first, because his duties consisted mainly of patrolling at about 10,000 feet. One day, however, he was attacked by the enemy and compelled to turn rapidly in the air; this caused him to vomit severely. He was so ill that he could not look about him, and the enemy were on his tail, firing into his machine before he realized what had happened. Two of his companions saved him and drove off the Germans. On coming down safely he was obliged to go to bed for two or three hours. He went up three times after this adventure, but vomited on each occasion and was sent home.

As a child he disliked swinging, and was easily made sea-sick. At the age of 14 he was swung upon a gate and was so sick that he was obliged to go to bed.

I saw him on his return from France. He was well built, strong, and healthy. He seemed so completely normal that I took him to the Candidates Board, and the examiners kindly put him through all the tests for me without asking any preliminary questions. He passed without qualification.

When, however, he was rotated in the chair the general reaction was excessive, although the visible response was normal in character. After ten turns clockwise, he rose and deviated to the right; the room seemed to be moving in a direction opposed to that in which he had been turned. After a pause of ten minutes, he was rotated counter-clockwise; he then staggered to the right and to the left unsteadily, whilst the room seemed to go round with the hands of the clock. This response was not abnormal, but the general reaction was disproportionately great. He was attacked with profound nausea, his face and hands sweated, and he said he felt exactly as he does in the air.

He was a highly-trained scientific student and was therefore given employment in the Technical Department. In the course of his work he frequently goes into the air as a passenger, and suffers from no discomfort so long as the flight is steady. But on one occasion recently he was up in a D.H. 9 with a B.H.P. engine, which gives off unpleasant fumes near the observer's face; the air was 'bumpy', and the combination of the two conditions caused him to vomit.

This is an example of a perfectly normal man, who has never been able to adapt himself to disturbances of equilibrium. The actual response to rotatory stimuli was normal in character, but the reaction it evoked was excessive.

The next case is one where the profound reaction to change of posture was one aspect only of a congenital want of nervous control, in a man of unusual physical strength and athletic ability.

Case No. 4. 2nd Lieut., aged 23¾.

He served for three years in the Infantry, and gained his commission from the ranks. He was splendidly built and had been a professional football player; but he had always noticed that if he looked up as he ran, he became giddy and tended to fall.

It is interesting to notice that, in spite of his build, appearance, and fighting record, he had always been 'nervous'. As a child he could not sleep without a light and was terrified of the dark. He always dreamt unpleasantly, and on waking 'saw big ugly faces' in the room. This occurred frequently up to the age of 16. During the last twelve months his unpleasant dreams had recurred; they mostly took the form that he was with the Infantry, and that 'they were in a tight place'.

After joining the R.A.F. he noticed that he did not suffer in the air so long as he flew straight, but turning made him giddy. He was most uncomfortable when the nose of the machine was put down, but banking always upset him. After several trials he was finally rejected as permanently unfit for pilot or observer.

This man's behaviour to the usual tests was in striking contrast to those of No. 3. He could not support himself for 15 seconds on either foot with the eyes closed. He failed altogether to balance the rod on the flat board, either with the eyes open or closed. But he showed equally gross defects to the respiratory tests, and could not support 40 mm. of mercury for more than 30 seconds; his pulse-rate behaved in a normal manner throughout, showing that this failure was not due to any cardio-vascular inadequacy.

From previous experience of such cases, I suspected that he might react violently to rotation, and I therefore turned him five times only in each direction; an interval of five minutes elapsed between the two observations. On being rotated he became so giddy that he sat coiled up clinging to the arms of the chair, unable to move. He did not even attempt to rise, but said he felt extremely ill, exactly as when he was in the air.

This is an example of an excessive reaction to sensory stimulation, due to lack of central control. In spite of his unusual physical development, this man was endowed with a psychopathic temperament, and the response to unusual stimulation of the vestibular apparatus was manifested in the form of a minor anxiety neurosis.

The next case shows that a pupil who starts with giddiness and vomiting in the air may overcame his disability and develop into an able pilot. But anything which diminishes general control may cause him to regress to his original condition.

Case No. 5. Lieut., aged 22.

After 3 years and 11 months in the Infantry he joined the R.F.C. During his first dual flight on an Avro he felt sick and vomited. He recovered entirely, quickly gained his wings, and went to France to fly Sopwith Camels. He had no giddiness, nausea, or other discomfort, even when 'stunting'.

But, after 90 hours war flying, he was shot down in aerial combat and chased from 9,000 feet by his opponent. On reaching the ground he found himself unhurt, but pinned under his machine, which had turned over.

Six days later he resumed flying and discovered that he had lost all confidence. He flew for 20 hours, but could not face the fighting and was sent home. After two months' rest he seemed to have recovered entirely, and was posted as instructor to a Training Squadron at home. He went up three times with the Wing Instructor and on each occasion vomited violently.

As a child he disliked swinging, and felt 'dizzy' and 'sick'. On a railway journey he cannot sit with his back to the engine without feeling 'squeamish'. All the members of his family react in the same way, both to swinging and to travelling by train.

The only one of the general tests which he carried out badly was balancing the rod with the eyes closed; he succeeded on the third attempt only with the right hand, and with the left failed altogether. He showed no excessive reaction to rotation; when turned ten times clockwise or counter-clockwise he deviated in the opposite direction, exactly like a normal person, but recovered himself quickly. The room seemed to be moving horizontally opposed to the direction of rotation. He did not suffer from nausea or any discomfort.

This was an instance when, in spite of an hereditary tendency to giddiness and nausea, the pilot adapted himself to the new conditions perfectly. But, with the shock of being shot down, he regressed, and all his old disability returned in spite of the fact that he was physically in perfect health.

In the following example the patient had a natural tendency to vomit in the air, but gained sufficient control to fly as an observer for more than three months in France and to take part in much aerial fighting. He suddenly regressed in consequence of acute fear.

Case No. 6. 2nd Lieut., aged 19.

He began his training in the air as an observer in April 1918 on a D.H. 4, and vomited three times in six flights. The nausea started about ten minutes after he left the ground with 'a drawing in the stomach up to the throat'; and as soon as he experienced this sensation he knew he must vomit. When it was over he felt better, and could go on flying comfortably.

The nausea came on apart from any aerobatic evolution; but when rolled, looped, or spun, he was 'dizzy' and 'sick' all the time.

He went to France as an observer and flew for $3\frac{1}{2}$ months in a Bristol Fighter, under war conditions. So long as he was not heavily 'stunted', he enjoyed his work and did not vomit. But, of all evolutions, rolling upset him most.

At the end of August 1918 he suddenly went to pieces under the following circumstances. His squadron was going to take part in a combined attack during the afternoon. In the course of the morning a new pilot went up for a turn in the air and dived into the earth on the aerodrome, killing himself and his observer. Shortly afterwards a second pilot and observer were killed in the sight of the patient and his companions. But the pre-arranged military operation could not be postponed, and he was obliged to go up with his squadron the same afternoon. He was intensely frightened and vomited in the air. From that time onwards he was sick whenever he went up, even when flying straight; finally he was so ill that his pilot brought him back shortly after starting.

He could never swing as a child, because it made him giddy, and he always vomited in a train, and sometimes even in a tramway car. When

uncomfortable in the air his sensations were exactly those he experienced in childhood before he was sick in the train. He had often suffered from sea-sickness.

Physically he was in perfect health, and was not tremulous. He passed all the usual balancing tests, and had no difficulty in supporting 40 mm. of mercury by blowing for 40 seconds. The pulse did not react abnormally to this form of effort.

But when rotated in the chair his reaction was characteristic. Turned ten times clockwise, he rose, stood with his feet apart, leaning forwards, shaking. He did not deviate, but moved slowly and unsteadily directly forwards to the table. He said that the room was not going round, but that his 'feet seemed to be rocking'. 'I had the drawing feeling in my stomach up to my throat, that I know so well in the air.' After a pause of ten minutes he was rotated in the opposite direction with exactly the same result. He stood unsteadily, in the same attitude, with his feet apart, quaking as if in fear.

Here, a healthy young man, relatively over-susceptible to disturbances of equilibrium, succeeded in adapting himself sufficiently to carry out his work as an observer under war conditions. But, with the development of an anxiety neurosis, due to an acute state of fear, the physical reaction became severe and overwhelming.

In the following case the patient had never experienced any discomfort until he attempted to fly after an attack of influenza. He then became giddy and vomited repeatedly in the air.

Case No. 7. 2nd Lieut., aged 19½.

He thoroughly enjoyed flying from the first, and had never had an accident. He obtained his wings without difficulty and was preparing to go to France, when on June 30, 1918, he had an attack of influenza. He was in bed for ten days with no complications and then went on leave.

On July 27 he went up for the first time since his illness as a passenger and vomited at 1,500 feet. 'I was giddy; my eyesight seemed bleary and full of specks, and it was like a bad bilious attack.' He was so determined to conquer this feeling that he went up again the same day and again vomited. This was a simple straight flight in an Avro.

As a child he thoroughly enjoyed swinging, and he has never been sea-sick, even when fishing from a small boat.

I saw him at the beginning of September 1918 and he was then easily tired, even by a short walk. He had lost a good deal of weight since the attack of influenza, but there were no abnormal physical signs in the lungs or heart. The balance of his hands was poor, and he was unsteady when standing on one foot. He had considerable difficulty in raising the rod on the flat board, especially with the eyes closed. But the respiratory tests were fairly well carried out [U-tube 45 secs.], and the pulse did not react abnormally to effort.

When turned ten times clockwise, he rose, deviated slightly to the left, but touched the spot on the table without difficulty. The room seemed to be revolving in the direction opposite to the rotation. He said he felt giddy, and looked it. The reaction, when turned ten times counter-clockwise, was exactly analogous; he deviated to the right and the room seemed to be going in the same direction.

In neither case was the visible response abnormal, but its effect upon his general condition was excessive. Throughout the rest of the day he felt

sick; his appetite was not impaired, but he had a continuous feeling of nausea. He also complained of an abnormal sensation, as 'if something was pressing down in the back of my head'.

This seems to be a case where the response to rotation was not abnormal; but owing to the diminution of general resistance, due to influenza, normal sensory impulses evoked an unusual general reaction.

The next case illustrates how a flying officer, who had never known discomfort in the air, may become giddy and vomit as a sequel to a gastro-intestinal disturbance. It also shows the close relation between the two conditions 'vomiting' and 'fainting' in the air.

Case No. 8. 2nd Lieut., aged 29.

He began to fly in Canada in January 1918 and enjoyed it extremely. In June he was posted to a squadron in England, and whilst there, during the first week of August, suffered from a short attack of diarrhoea. Throughout the four days of its duration he continued to fly, but felt so weak that he was forced to use two hands to the controls on steep turns; suddenly, at about 1,000 feet, he was attacked with 'dizziness' and thought he was about to vomit. After resting for a few days, he seemed to have recovered entirely and took two short flights without ill effects.

He was then posted to another squadron and began to suffer from indigestion, with 'wind', 'cramps in the stomach', and 'some pain shortly after taking food'. He was given a laxative, and this started another attack of diarrhoea. He did not fly for a week, but on September 8, 1918, after 20 minutes in the air, he became 'dizzy'. His head seemed to 'swim', his vision became hazy, and at about 100 feet everything 'went black' for a few seconds.

After a good night's sleep he went up again next day and was perfectly comfortable, so long as he flew straight; but as soon as he began to circle round a ground target he became 'dizzy'. 'The target started going round with me.' He felt sick, broke out into a sweat, and repeatedly put his head over the side, partly because he was certain he was about to vomit, and partly in the attempt to obtain fresh air. He kept on swallowing and felt all would be right if thereby he could prevent something coming up from his stomach.

He has never been sea-sick, and could swing with pleasure as a child.

I saw him in October 1918. He was still suffering from flatulent dyspepsia; his bowels were opened once or twice daily, and the motions were always somewhat loose. The tongue was clean, the teeth were fairly good, and there was very slight gingivitis. There were no abnormal physical signs in the heart or lungs, but he had distinctly lost weight. He slept well, and did not dream unpleasantly.

He passed all the tests for balance without difficulty, but rotation in the chair produced a characteristic reaction. On turning him ten times clockwise, he rose, swayed with eyes half closed, and appeared dazed; but he stepped forwards and touched the table without deviating definitely in any one direction. He said: 'Things seem to start going to the right and yet I seemed to be able to control it by blinking. Everything went misty just as it does in the air, and my stomach felt funny.' On rotation in the opposite direction, he rose slowly and staggered to the right, pulled himself together and moved to the left, finally touching correctly. He said: 'This time it was more as if I could not get where I wanted and misjudged my

distance. I thought I was right on the point, when I was a long way off. This is just like my landings after an attack in the air.' He added that he felt 'squalmy' in the stomach.

In this case the abnormal reaction took at one time the form of giddiness and nausea, and at another time that of partial unconsciousness; it forms a transition to the next group, where the pilot is said to 'faint in the air' at low altitudes.

(*d*) '*Fainting*' *in the Air.*

In the previous section I have described a series of cases where the reaction to disturbances of equilibrium took the form of giddiness and nausea. Another not uncommon abnormality is what is usually called 'fainting' in the air. A 'mist' or 'haze' comes before the eyes and 'all goes dark'. It is not an unpleasant sensation, and is said to resemble closely the early stage of an anaesthetic. This passes into complete insensibility and the pilot may fall forward on to his controls; but if he is strapped in sufficiently tightly his hands leave the stick and his feet no longer act on the rudder. Should he be spinning on a stable machine, he may come to himself some miles away flying on a level keel.

Case No. 9. 2nd Lieut., aged 20.

He began flying in February 1918, and obtained his wings in July of the same year. From the first moment he thoroughly enjoyed being in the air, and flew well without discomfort or difficulty.

But, in the middle of July 1918, he was in bed for two days with a short attack of influenza. Four days later he was flying an R.E. 8 when he 'felt faint' at 5,000 feet. He came to himself at about 1,500 feet, to find he was in a spin; he managed to right the machine, but broke an axle on landing.

I saw him in October 1918, after a prolonged period of leave and ground duty. He was then a healthy young man of rather poor physique. Heart and lungs were normal. He could sustain the mercury in the U-tube for 40 seconds. During this test his pulse volume became rather small and the tension poor; the rate, however, was in no way excessive, and recovery occurred within a normal period after the close of the effort. The balance tests were carried out perfectly.

Rotation produced a normal response. On turning ten times clockwise, he rose, deviated slightly to the left, but touched the spot on the table easily. He said he was giddy and that the room seemed to revolve against the hands of the clock, but he was not in any way uncomfortable. Turned in the opposite direction, he deviated slightly to the right; the room seemed to go the same way, but he recovered rapidly. He suffered from no nausea, headache, or other discomfort.

This is the case of a man of somewhat poor physical development who 'fainted' in the air after an attack of influenza.

The next example of this condition is a pilot with long experience and 250 hours war flying in France. He became chronically tired and, at the end of a long patrol, 'fainted' in the air.

Case No. 10. Capt., aged 20½.

He joined in August 1916, and went out to France as a pilot in December of that year.

In July 1917, after 250 hours flying under war conditions, he was returning from a long patrol when he 'fainted' in the air. But, knowing he was going on leave, he said nothing about it and came home on July 28.

After three weeks he was posted to a Training Squadron as instructor. He began to notice that he had lost all keenness for flying and that he 'started at a noise'. One day in December 1917 he was suffering from neuralgia in his face and went up in a D.H. 6. He 'came over foggy and silly', and became unconscious at about 4,000 feet. He 'woke up' at about 1,500 feet and flew the machine back to the aerodrome without difficulty.

He then went to hospital for a fortnight and, after three weeks' leave, returned to his squadron feeling perfectly well. He was extremely keen and thinks he never taught better; he instructed thirteen pupils in a month. He was then transferred to another aerodrome, where he remained for four months. At the end of that time he found he could not stand 'stunting'.

In July 1918 he was given ground duty. He came before me in November because he was anxious to return to flying. He could walk 20 miles without discomfort, and had been playing two rounds of golf daily. At first he noticed that he was off his game; his normal handicap had been 4, he was now playing 8, though improving daily.

When I saw him he was in splendid physical condition, and passed all the respiratory and balance tests perfectly. On opening his eyes, after ten turns clockwise, the room 'seemed to take half a turn to the right and then stand still'. He rose, walked straight to the table and touched well. He added, 'It was just like coming out of a cloud; I felt I was going to pull out at once.' On being rotated counter-clockwise, his sensations were *mutatis mutandis* identical. He experienced no discomfort of any kind.

Evidently this was a case where 'fainting in the air' was associated with exhaustion, consequent on the strain of excessive flying under war conditions. It is interesting to record that this pilot had never 'crashed' since he obtained his wings.

The next case shows that 'fainting in the air' may be the terminal phase of a long period of loss of confidence.

Case No. 11. Capt., aged 27.

This pilot flew before the war in a training school, and was given his wings when he joined up in January 1916.

On July 20 of the same year he suddenly lost confidence under the following conditions. When taking a machine across country he was compelled to make a forced landing in a field. He tried several times to get out, but could not clear the surrounding trees. On landing, after the seventh attempt, he noticed twigs from the trees in the tail and under-carriage of this machine. This brought home to him how near he had been to disaster, and he became so frightened that he left his aeroplane and returned by train. Next day he tried to fly, but failed twice; every little bump 'set my heart going, and I used to wonder if I should come down again'.

He was discharged in August 1916; but in December he began to fly again as a test-pilot. In March 1917 he was given a commission in the

R.F.C., and flew so confidently and well that he was made Flight Commander to a Training Squadron.

On October 27, 1917, he went to France in charge of a section for issuing machines. On December 22 he was testing an S.E. 5, when he 'fainted' at about 5,000 feet. 'Everything seemed to go black all at once.' He recovered himself at about 1,000 feet, but lost consciousness again. He next remembers that he was on the ground, hanging upside down from the machine, which had turned over. He could not release his belt, but was not injured in any way.

After a short stay in hospital, and some months of ground duty, he began to fly again at the end of April 1918. He then became sleepless and frequently suffered from nightmares; these consisted of a recurrent dream that he saw a machine falling without wings. He was never the victim of the accident, but always seemed to be watching some one else falling in this manner.

When he went into the air he felt 'timid' and, if the air was still, he became drowsy. When this sensation came on he felt exactly as if he was 'sitting in an easy chair in front of a warm fire and falling off to sleep'. The 'fainting' came on suddenly, but the onset of the 'drowsiness' was gradual.

He sometimes had this feeling when he played lawn-tennis or billiards, or when he concentrated his attention on a book. We set him to play lawn-tennis under observation, and he was certainly unnaturally slow. When he wanted to take a backhand stroke, he could not bring his racquet across quickly enough to hit the ball. He complained that he found exactly the same difficulty in the air; he could not 'correct bumps', and was 'slow in adapting'.

Up to the time when he 'fainted' in the air he knew nothing of the abdominal sensations experienced by many persons when they descend in a lift. But he then began to be conscious of the 'feeling in the stomach', not only with any small displacement in the air, but also when going down in the lifts of the Tube Railway. He is certain that he never experienced this sensation of 'bumpiness' until February 1918. Since then he has never been free from it with the smallest drop or vertical bank. Moreover, when he had finished a spiral turn, the horizon continued to rush by for a long time afterwards in the direction in which he had been turning. This was another new experience.

When I saw him in August 1918 he was a well-built man of normal appearance, with no tremor of the hands or other signs of disease.

He walked a line and turned badly. He supported himself with difficulty on one leg and could not stand for more than 10 seconds on either foot. He failed entirely to balance the rod on the flat board with his eyes open or closed.

After ten turns in the chair he deviated strongly in the opposite direction to that in which he had been rotated, and did not reach the table at all. He staggered and fell into my arms as if drunk, and said he could not see anything distinctly enough to say in what direction the objects in the room seemed to be moving.

(*e*) *Comparison of the two reactions, 'Giddiness' and 'Fainting' in the Air.*

Any normal man can be made to suffer profoundly in the air if the rotatory alternations are sufficiently intense, and particularly if the head is held in some abnormal position. For example,

to look upwards at the top plane during a rapid spin is a certain means of inducing giddiness and nausea. In the same way, Major V. B. could provoke at will the preliminary symptoms of 'fainting' by executing evolutions which produced unusually intense rotatory stimuli.

Such reactions are not in themselves abnormal; but, in the examples I have quoted, they could be evoked with undue ease and were unusually violent. Sometimes the disturbance, due to changes in equilibrium, assumed the character of a true vertigo, accompanied by nausea and vomiting. In other cases it consisted of a withdrawal of consciousness, usually spoken of as 'fainting in the air'; this does not begin with a true vertigo, but with a feeling like that of passing under an anaesthetic. Surrounding objects grow distant, the 'horizon seems dark', and the actual loss of consciousness may be preceded by a drowsy state, in which it is impossible to act. The final event, as far as the pilot is concerned, depends on the stability of the machine. If it is of a type that passes out of a spin naturally on releasing the controls, the pilot may come to himself to find that he is flying far away from the point at which he lost consciousness; or he may have dropped several thousand feet. On the other hand, with many quick scout machines, which require an active effort before they cease to spin, the result is not uncommonly a more or less severe 'crash'.

Both the true vertigo and the loss of consciousness are the direct consequences of changes in equilibrium. What, then, are the underlying causes of this diversity of reaction? From an analysis of fifteen cases of 'giddiness' or 'fainting' in the air, where I was able to subject the pilot to exhaustive examination, I should like to put forward the following solution.

Out of ten pilots and observers who came under my care for giddiness and vomiting, all but one suffered in this way when they first went into the air; many of them succeeded in adapting themselves more or less successfully to the new conditions, until some factor arose which led to diminished control. They then regressed to the earlier reaction, and adaptation was no longer possible. It is interesting to notice that all nine patients had been unable to swing with pleasure in childhood, and many of them had been habitually sick in the train.[1] As they grew up, they gradually conquered this childish reaction; but, on being exposed to changes of equilibrium in the air, it returned. In most cases it was again brought under control, reappearing, however, with any cause which lowered the general resistance of the central nervous system. In the one case where the pilot had not suffered in the air during the early stages of his training, he

[1] It is interesting to notice the close association of rotatory vertigo and train sickness. For it is obvious that the latter is evoked from the visual apparatus, whilst the former is due to vestibular stimulation. The reaction is identical in spite of the diverse nature of the peripheral excitation. Another example of this order is the giddiness and nausea induced by rotating the walls of the room so that the victim, seated on a chair at rest, obtains an illusion of movement. One of the principal functions of the vestibular apparatus is to maintain the balance of the eyes; if this is disturbed, giddiness will follow although the semicircular canals have remained at rest.

began to vomit after an attack of influenza; this produced severe loss of weight and was followed by a constant feeling of fatigue. He had been, moreover, an unusually nervous child, and was not able to sleep without a light until he was sixteen years of age.

Giddiness and vomiting in the air are an expression of undue susceptibility to changes in equilibrium. This is usually constitutional; it may be so severe that it can never be corrected (No. 3), but in most cases adaptation is possible. The tendency to be giddy in a swing or to vomit in a train are overcome on the passage from childhood to adult life; in the same way, the reaction to disturbances of equilibrium may be controlled with the gradual acquisition of facility in the air. But any disturbance, physical or mental, associated with diminished general resistance in the central nervous system, will cause regression; the patient reverts to his original mode of reaction.

On the other hand, none of the five patients who 'fainted' in the air suffered from any discomfort on learning to fly; all enjoyed it from the first. Not one of them had ever been giddy in a swing or suffered in childhood from train-sickness. Evidently they were endowed with the power of controlling the effects produced by changes of equilibrium on the vestibular apparatus; vertigo, with its accompanying nausea, was absent. But when the impulses from the semicircular canals became too violent to be held in check they expended themselves by producing a withdrawal of consciousness. This begins with a narrowing of the field of attention, which may culminate in complete oblivion. Such a reaction may make its appearance in normal pilots, provided they push the rotatory stimuli up to unusual limits, as was the case with Major V. B. during his technical experiments. But under the conditions of normal flight fainting in the air at low altitudes is always evidence of diminished general resistance. Sensory impulses, which should be controlled, become capable of evoking an abnormal general reaction. This may be due to stress of war-flying (No. 10), to fear and anxiety (No. 11), to some gastro-intestinal condition (No. 8), to the after-effects of influenza (No. 9), and probably to a multitude of causes which act in a similar manner.

But amongst the group of pilots who faint in the air, the natural resistance to vertigo is high; the impulses evoked by disturbances of equilibrium do not show themselves, therefore, in giddiness and nausea, but in a disturbance of consciousness.

This difference in reaction to changes of equilibrium in the air can be demonstrated by observations on the rotating chair. The effect produced upon those who were 'giddy' in the air was in sharp contrast to that produced in the group who had 'fainted'. Of the ten who had been 'giddy' and vomited, nine reacted excessively to rotation in the chair; they became more or less giddy, sweated, and suffered from nausea. The actual form assumed by the physiological response was not abnormal, but it induced an excessive general reaction with undue ease. In one instance only was this not the case; he had been shot down in aerial combat and his regression was assuming an almost pure psychopathic form.

On the other hand, of the five pilots who had 'fainted' in the air, three suffered from no discomfort of any kind after rotation in the chair; in two cases 'everything went misty'; and both patients complained that they felt exactly as they did in the air.

It would seem as if those who have a natural tendency to become giddy on rotation, react to disturbances of equilibrium with nausea and vomiting. Others can dominate the vertigenous aspect of the impulses from the semicircular canals; in them rotatory stimuli, if sufficiently severe, evoke the condition known as 'fainting'. How far the tendency of the reaction to assume this form depends on a predisposition to react to any severe shock by cardio-vascular disturbance, must be determined by future research. It may be found that even the 'fainting' induced by lack of oxygen is the same reaction of the central nervous system to stimuli of an entirely different order. My cases, however, are too few in number to afford an answer to this important question in applied physiology.

Wider experience may show that there are other forms of reaction which may occur in the air. Physiological responses are not inevitable and rigidly-defined manifestations. Such a case as No. 8 shows how closely related are the two reactions, 'fainting' and 'giddiness' in the air; for although the patient is classed under the first heading a violent dive on to a ground target induced nausea and many of the symptoms of 'giddiness'.

(*f*) *Attacks of minor Epilepsy in the Air.*

In the early days of the war, before we were familiar with the pathology of 'fainting' in the air, some neurologists attributed the condition to minor epilepsy. But further experience has shown that this must be an excessively rare cause of pathological states in the air. I have met with one undoubted instance only; but the symptoms form such a striking contrast to the commoner form of flying disability, that the case seems to me worthy of record.

Case No. 12. Sergeant-Pilot, aged 24.

He joined the R.N.A.S. in September 1914, and went to Egypt in June 1915. Very shortly after his arrival he was seen by the Medical Officer in an attack of minor epilepsy. He returned to England and was discharged on August 15, 1915, with this diagnosis.

He then learnt to fly at his own expense, and applied for a commission in the R.F.C.; he was, however, rejected on his previous history.

But in September 1916 he succeeded in joining without a Medical Board and, after passing through a theoretical course, went to a squadron as 2nd air mechanic. Here he rapidly learnt to fly, and gained his wings after about 20 hours in the air.

In March 1917 he went to France, and had his first and only severe accident in May of that year. He was flying a Sopwith two-seater. All he remembers is that, on rising 300 to 400 feet from the ground, he suddenly felt his arms and legs growing 'powerless' and he lost consciousness. He does not remember hitting the ground; but not more than a few seconds later he climbed out of his machine and helped his observer, who was hurt.

He had no conception of what had happened, and says he felt exactly as if he were a spectator, who had come to the rescue.

He did not fly for three weeks and then went to another squadron in France as a test pilot; there he remained for six months. He had no trouble in the air, but one evening, when walking with a friend, he suddenly became unconscious and fell to the ground.

In March 1918 he came to England and acted as ferry pilot; but he had many small attacks on the ground, and in September had a severe seizure in the street. He fell and became absolutely unconscious; he remembers walking on the pavement and then found himself in a tramway car with 'people fussing round' him.

He has been subject to these attacks ever since he was about 14 years of age. He 'suddenly loses count', becomes dazed, and then recovers to resume his work as if nothing had happened. When in the street, he has dropped things from his hands and walked on oblivious of his loss. He has never wetted himself or bitten his tongue.

In 1911 he had an attack whilst riding a motor bicycle. This led to a bad spill, and he severely injured three fingers of his left hand.

He enjoys flying intensely, and never feels better than when he is in the air. A long period of ground duty makes him depressed, and leads to a considerable increase in the number of the attacks. He has had one 'crash' only; but sometimes on long journeys, when acting as a ferry pilot, he has 'felt queer' in the air. He has always been fortunate 'to be high enough to come to myself all right, though I have lost height several times'.

One brother and one sister have similar attacks, and his father, who died of pneumonia, drank very heavily. The patient himself is a teetotaller.

He was a well-built rather nervous-looking man. His heart was normal, and the pulse showed a perfect reaction to measured effort, returning with normal rhythm in one minute and a half.

His hands showed some irregular tremor, and his tongue was not steady. He could stand on either foot for 15 seconds on the first attempt, but could not balance the rod on the flat board, either with the eyes open or closed.

The pupils were of medium size but oscillated widely when his eyes were fixed on a distant point. The reflexes were normal, and there were no signs of gross disease in the nervous system.

This case differs from all those previously described in this chapter by the fact that the attacks occurred before the patient took up flying. Moreover, the majority happened on the ground and were associated with the usual manifestations of minor epilepsy. With care it should not be difficult to exclude even epileptics of this class from the Flying Services.

(*g*) *Conclusions.*

Giddiness, vomiting, and fainting in the air may occur from many different causes apart from physical or chemical deficiencies in the atmosphere.

Candidates who show any signs of disease of the ear, nose or throat, are excluded from the flying branch of the Air Force. But the most careful examination cannot ensure that the pilot will not suffer from inequalities of tension in the middle ear on rapid changes in altitude. This is particularly liable to

occur if a cold or sore throat leads to partial or complete blocking of one or other Eustachian tube. This temporary change in the conditions of tension, external to the labyrinth, is a potent cause of giddiness, especially in scout pilots engaged in war-flying. It is characterized by the fact that it continues for a time on the ground after landing and is frequently associated with pain (Sydney Scott).

But there are many cases of giddiness and fainting in the air which bear no relation to abnormal conditions of those portions of the auditory apparatus external to the receptive nervous mechanism.

Any normal man can be made giddy during aerobatic evolutions, such as spinning, if he holds his head in certain unusual postures. We can bear with impunity certain violent disturbances of equilibrium if the stimulus is expended on certain parts of the vestibular apparatus, but not if it falls on other combinations of the semicircular canals. In the same way, provided a normal pilot is not unduly susceptible to giddiness, he can induce at any rate the preliminary stages of fainting in the air by pushing rotatory stimulation experimentally to abnormal limits.

But machines are so constructed, and pupils are taught to carry out aerobatic evolutions in such a manner, that the majority of normal men do not suffer from these symptoms in the air. There are, however, some in whom the physiological response is so violent that it can never be overcome, and they are always giddy and nauseated whenever there is any sudden or considerable change in equilibrium. Others suffer at first, but may overcome the reaction as they acquire facility in the air. Such persons are liable to regress under the influence of any condition, physical or mental, associated with diminished central control.

The majority of these patients could not swing with comfort in childhood and habitually suffered from train-sickness. When rotated on a chair the larger number were uncomfortable and suffered from nausea and sweating.

On the other hand, the pilots who came under my observation for 'fainting' in the air had all enjoyed flying from the first. They had experienced no discomfort in childhood, either when swinging or when travelling in the train. In two out of the five cases I was able to evoke that 'mistiness', which is one of the preliminary stages of fainting, by rotation in the chair; the others showed no abnormal reaction to this method of stimulation.

Epilepsy is one of the rarest causes of 'fainting' in the air. One definite case only has come under my observation during the later stages of the war, since the institution of a strict medical examination for admission into the Air Force. This is reported on p. 29. Differential diagnosis should not be difficult, for in this case, as might be expected, the larger number of attacks occurred on the ground under conditions that excluded any form of vertigo.

D. Sensations experienced by Normal Persons in Machines heavier than Air.

(*a*) *Introduction.*

Balance in the air or on the ground depends primarily on the character and intensity of many diverse sensory impulses. Changes in the posture of the body as a whole affect the vestibular apparatus, the spacial aspects of vision, and the afferent mechanism of joints and muscles. If these various sense-organs function normally, the basis is given for those actions necessary to ensure stability, whether on the ground or in the air. Any external condition which produces an abnormal series of responses in any part of this complex sensory mechanism will lead to defective equilibrium. Giddiness, due to unregulated tension in the middle ear, and the disastrous effects of heterophoria are examples of such aberrant stimulation of normal sense-organs.

If want of balance were merely a matter of irregular external stimuli, or of some defect in the peripheral sense-organs, the question would be easy of solution. But I have been able to show that an abnormal reaction may be caused not only by faulty sensory impressions but by lack of what, for want of a better word, may be called 'higher control'. A man's 'muscular sense' may be perfect; and yet, when his eyes are closed, he may be unable to stand on one leg or to balance a rod on a flat board. His vestibular response, when rotated on a chair, may be normal, but the sensory impressions evoke nausea or the preliminary stages of fainting. Two factors must be considered in all observations on sensation, the character of the primary response and the nature of the final reaction.

Moreover, physiologists have long been familiar with the fact that a constant physical stimulus does not evoke in every case an identical physiological response. More mechanically-minded investigators are accustomed to assume that a strictly regulated physical stimulus must be followed by a directly proportionate functional response. On this hypothesis a body at a certain temperature should always produce an identical sensation of heat or cold; but this is notoriously not the case. A temperature of 30° C. may seem at one time hot, at another cold, according to whether the hand has been previously soaked in cold or in hot water.

Again, the impression produced by a physical stimulus, however carefully selected, is not of necessity simple, but is frequently extremely complex. It may evoke impulses which are incompatible with one another from the sensory point of view. Thus a temperature of 45° C. stimulates the heat-, the cold-, and the pain-spots; normally it produces a sensation of heat. If the mechanism for the reception of heat-stimuli is absent, 45° C. seems to be cold; if the part is insensitive to both heat and cold, pain alone follows the application of this temperature although it normally excites pleasant heat. In this case a physically simple stimulus evokes complex and incompatible

impulses, which must be sorted and integrated before they can form the underlying basis of a sensation.

Even then the struggle is not over. For it may happen that the mind is not at the moment attuned to accept a sensation of this kind; it is therefore suppressed, and does not enter the focus of consciousness. Under such circumstances some other group of sensory impressions may become dominant, to the exclusion of those more naturally anticipated. For example, we all know how certain forms of toothache are relieved by taking food. The physical cause of the pain is not influenced by the mechanical act of deglutition; but the neuralgia is inhibited for a time by the massive impulses poured into the central nervous system from the upper gastro-intestinal tract.

An identical stimulus can evoke in the same individual a varying response; what wonder that the action of the same physical forces may produce the most diverse effects upon different people. A good example of such differences, drawn from daily life, is the sensation produced by descent in a lift. Many people experience a peculiar 'sinking in the stomach' or 'feeling as if my stomach was being left behind' when moving downwards. Others have never been conscious of this sensation. unless the lift moves with a rapidity unusual in this country. Between these two extremes lie those who recognize this 'feeling' if the movement starts somewhat suddenly, but not under ordinary conditions. This sensation, like giddiness in the air, can be acquired. One young pilot knew nothing of any such 'feeling in the lift' until after an attack of influenza; the first time he travelled on the Tube Railway, on recovering from his illness, he noticed that the descent of the lift produced a peculiar effect he had never experienced before. When he returned to duty ten days later he found he had lost all confidence in the air and was 'gripping his controls tightly and tensely'.

Thus between the impact of the physical stimulus on the sense-organs and the final production of a sensation, lie innumerable possibilities of variation in the physiological and mental reactions. What wonder that the sensations induced by flying a machine heavier than air differ fundamentally in different persons, though the stresses and strains may be identical.

These individual differences start from the moment the machine leaves the ground. In some the uncomfortable bumping, caused by 'taxy-ing' across the aerodrome, gives place immediately to a feeling of profound security. If the passenger is strapped in with a well-fitting belt and his pilot is expert in correcting 'bumps', the machine seems to be the stable centre of the universe; it is the earth and sky that shift and move. A vertical bank produces no sensation beyond the feeling that the country with its roads, woods and houses is tilting in a peculiar and definite manner. Even during a flat spin it is the horizon that races round like the rim of a rotating bowl. An expert pilot talks in terms of a moving universe; he says, 'I came out to find the earth rushing up and so I pulled my stick back'; or 'My horizon was crooked, so I knew I was flying one wing down'.

In those who are fortunate enough to experience this stability from the first, it transcends any security obtainable on earth or sea. But many first-rate pilots have been frightened and unhappy on their first flight; they cannot believe that any one felt secure from the first moment he left the ground. With them confidence is acquired. But since it has been gained by practice it can also be destroyed by any condition which lowers bodily or mental vigour; and this is the case, not only with those who have adapted themselves to conditions in the air, but also with those in whom such confidence is innate.

Any experience which induces anxiety or fear may destroy the confidence of even the finest pilot. To be shot down in aerial combat, or to land in flames, is sufficient to upset the sense of security in any one, and may lead to subsequent want of adaptation to changes of equilibrium in the air. But should the concussion produced by the 'crash' abolish all memory of the events directly preceding the accident confidence may remain unimpaired. For this reason some who have passed through a terrifying experience can subsequently fly as well as ever. It has left no trace on the memory; the whole episode, including perhaps events of some hours before the ascent, has been entirely wiped out.

On the other hand, the sudden realization of danger may destroy all feeling of confidence on the spot (No. 11). The pilot not only feels himself insecure, but may become clumsy in his actions and suffer from abnormal sensations in the air. The anti-aircraft guns of the enemy produced more harm by undermining confidence in the air than by the actual destruction of machines; for when a pilot began to dream of 'Archies', he was near the end of his flying career at the front.

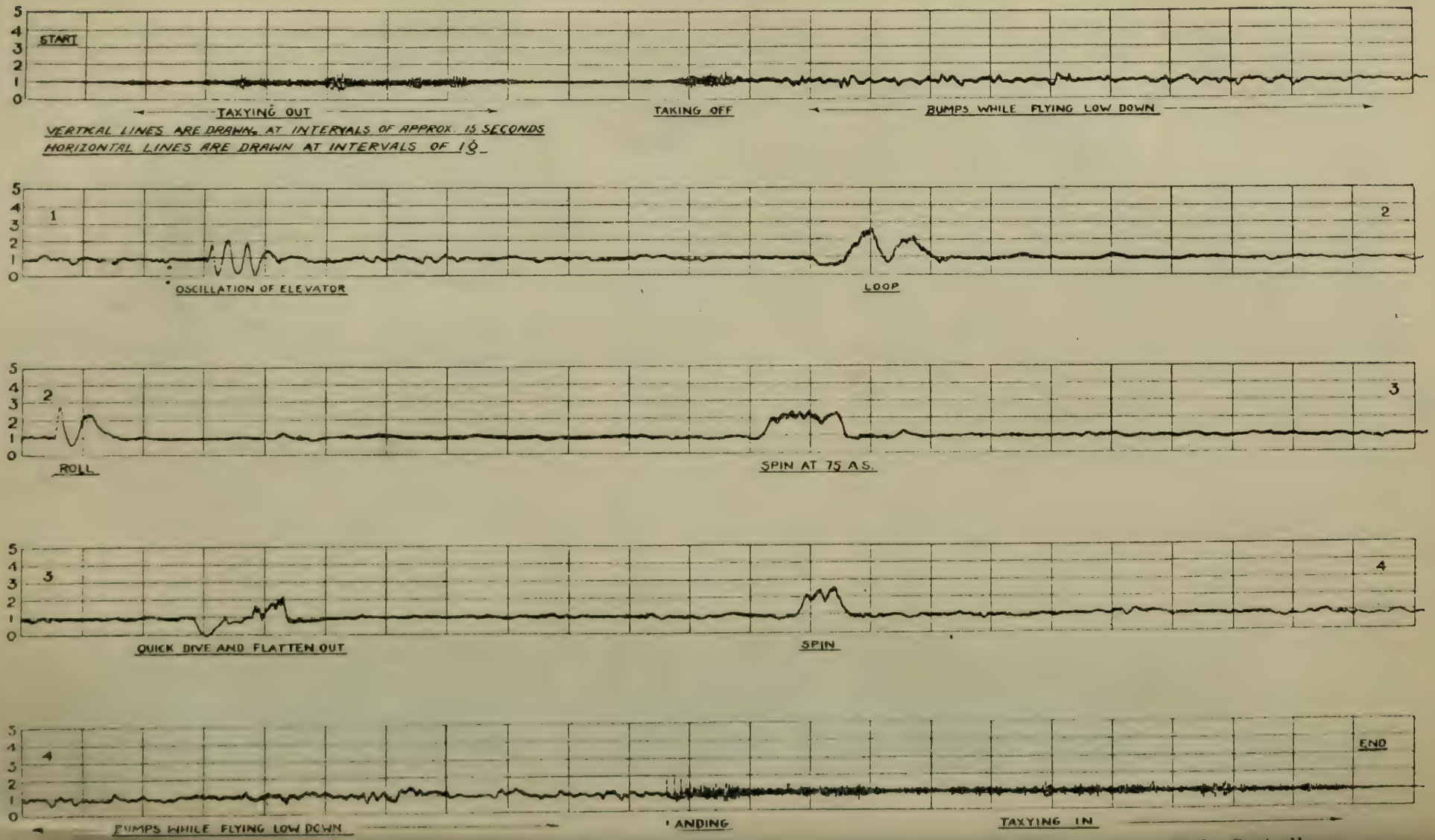
(*b*) *Actual Experience during Aerobatic Evolutions.*

It is a matter of everyday experience that the same physical stimulus does not produce an identical effect on different human beings. But the physicist finds it hard to believe that the strong mechanical forces in action during aerial flight may lead to no conscious effect; and yet from a physiological point of view this might have been exactly anticipated. The more perfectly the passenger feels himself part of the machine, the more he will tend to translate changes of posture into some alteration in the position of surrounding objects. Stresses and strains disappear so long as there is perfect physiological adaptation to changes in equilibrium. In this way we gain a rapidity of action which would be impossible if every physical alteration entered consciousness and necessitated a corresponding action of the will. A good pilot does not fly by rule nor does he depend entirely on his instruments. When he wants to 'bank' he says, 'I just lean over and it comes,' exactly as when rounding a corner on a bicycle.

The following figure gives the actual forces to which the machine is exposed during various evolutions, as shown by means

VARIOUS MANŒUVRES ON A BRISTOL FIGHTER

29-3-18



Committee for Aeronautics, by permission of the Controller,
H.M. Stationery Office.

of the accelerometer.[1] From this beautiful record it will be seen that a 'zoom' leads to a rapid drop to 0, followed by an irregular rise to 2 g,[2] just before the machine flattens out.

On looping, g first drops a little, then increases rapidly almost to 3, falls and rises again to about 2. A roll gives a similar but shorter rise, followed by a fall and a second flat-topped curve.

In spinning g runs up and oscillates round about 2, dropping quickly, when the machine is pulled out of the spin.

The actual sensations experienced by a passenger during these evolutions were determined independently by four medical observers. Colonel Birley, principal medical officer to the Headquarters of the R.A.F. in France, made a long series of experimental investigations from the back seat of a Bristol Fighter. These form the basis of the inquiries summarized in this chapter. To Captain Rippon, medical officer to the 29th T.C.S. (the 'Medical Flight'), I am indebted for much personal experience of flight under varying conditions. Mr. Sydney Scott has also given me valuable information, especially with regard to giddiness and analogous sensations. Lastly, I have myself experienced, in an Avro, the effect of all these evolutions.

The whole matter was then discussed with a series of pilots, particularly with Major Bird, D.S.O., and his colleagues of the 'Medical Flight', who are accustomed to observe their own sensations and to listen to those of their pupils. To them my sincere thanks are due for their patience, and for the remarkable way in which they worked out these problems for themselves.

Most pilots, however, are not good witnesses in such an inquiry, unless their reactions are in some way aberrant. A normal pilot is so busy flying his machine, and is so instinctive in his reactions, that he has neither time nor attention to observe his own sensations. On the other hand, the four medical men who went up as passengers were not only trained observers, but were intent on discovering the effect produced by each evolution in turn.

In contrast to the evidence yielded by these normal individuals stands that of Captain H., a most skilful pilot, who returned from France, after six months' flying under war conditions, owing to nausea and vomiting. He was engaged on

[1] Report on the Measurement of acceleration on Aeroplanes in Flight, by G. F. C. Searle and W. Cullimore, June 1918.

[2] g is the symbol used for the acceleration per second of a body falling freely under gravity.

The weight is the mass multiplied by g.

Forces are proportional to the accelerations they produce on the same mass. Thus we may express the relative magnitudes of the forces acting on a body in terms of the accelerations these forces are producing.

The record (Fig. 1) gives the resultant force acting on any mass within the aeroplane in terms of the ratio of the acceleration that force can produce to the acceleration (g) which would be produced by gravity, acting normally. Thus, when the force registered is 1, it is exactly equal to that of gravity.

The supporting force of the seat, when sitting normally in a chair, is equal to g; seated in an aeroplane it is equal to g, multiplied by the number on the record, the value of which depends on the nature of the evolution being carried out. It is usually spoken of as 'one, two, or three g'. The excess or deficit above or below the value of g may be considered the measure of the abnormal physical forces acting on the body.

mechanical experiments in the air for the technical branch, and gave valuable evidence before the Committee on Accidents, especially in answer to my cross-examination.

Lastly, my thanks are due to Major V. B., another officer engaged in technical experiments, who was able to produce fainting in the air by excessive disturbances in equilibrium. He supplied me with many interesting details.

(i) '*Zooming*'. During this evolution the machine is dived steeply and then rises almost vertically in consequence of the great acceleration of momentum. Sometimes on quick-flying scouts the preliminary descent may be eliminated; the essential part of the 'zoom' is a steep climb. This throws an enormous strain on the machine and exposes the pilot to violent changes in stress.

All the medical observers, however, failed to notice any sensation during this manœuvre, and the three pilots of the 29th Training Squadron were of the same opinion; but they added that there was a sensation 'as if you were loose on your seat', when flattening out on the top of the 'zoom'.

On the other hand, Captain H. insisted that he felt his 'head being squashed into his body'; 'you feel you are being forced into your seat and it is impossible to move your legs though you can move your arms.' Here we find the first flat contradiction in testimony. Four highly-trained scientific observers and three pilots chosen for their perfect adaptation to conditions in the air, failed to perceive any sensation until the end of the manœuvre. On the other hand, a skilled pilot, with technical knowledge of a high order, who suffered from nausea in the air, was acutely conscious of the physical forces acting on his body during this evolution. In fact he said, 'If I were to lose this sensation, I should be worried, because then I might be putting forces on to the machine which would break it'.

Major V. B., the technical pilot who could make himself faint by carrying out certain experimental evolutions, was in complete agreement with the majority of the observers. He said that during a 'zoom' he 'felt nothing except the joy of doing it'.

Thus it is evident that what we call perfect adaptation to disturbances of equilibrium in the air is associated with control by the central nervous system of the various afferent impulses, evoked by the action of intense physical forces on the human body. Should they be permitted to rise into consciousness and cause sensations during normal flight, the pilot tends to suffer from giddiness and nausea when they reach any considerable intensity.

(ii) *Looping*. None of the medical witnesses experienced any giddiness or definite sensations during an ordinary loop, and the three pilots of the 29th Training Squadron agreed with these observations. Colonel Birley noticed that, after the fourth or fifth consecutive loop, a sensation gradually came on of 'being loose in the seat'.

As might be expected, the account given by Captain H. again differs profoundly from that of the other observers. He feels that his head is being squashed into his body; as if he were

being forced into his seat. 'It is impossible to move your legs although you can move your arms freely.' This alternates with a feeling of 'decreased gravity'; 'it makes me feel as if my stomach was coming up. I sweat and may have nausea. I have put my head over the side but have never been sick.'

Major V. B.'s experiences are particularly interesting. He feels no sensation whatever during a perfect loop; but if he makes a short loop, a mist comes before his eyes like the early stage of going under an anaesthetic. If he makes five or six perfect loops consecutively he loses his horizontal and comes out to find he is flying wing down. 'You haven't the power to get her right unless you can get your head clear again. Something goes wrong after the third or fourth loop, which spoils their perfection.'

Here again we see the same difference in central control. In perfectly adapted persons simple looping produces no sensation, whilst Captain H. was sensible of the physical forces acting upon him throughout. These sensations came to Colonel Birley after a fifth or sixth consecutive loop only, and then in a mild degree. Major V. B. experienced nothing unless the loop was imperfectly performed, when he felt in a slight degree the preliminary sensations of fainting; a series of perfect loops disturbed his power of flying horizontally unless he made a definite corrective effort.

(iii) *Spinning.* The effect produced by spinning in normal persons depends greatly on the position of the eyes and head. Pilots vary profoundly in the attitude they adopt; some keep their eyes on the instrument board and do not look outside the fusilage, others fix some point on the ground. Many pilots contend that if the eyes are thus directed throughout 'you remain conscious of your position in space, but if you look inside the fusilage you don't'. Both are safe methods and should not cause giddiness in normal persons.

Colonel Birley, who has made many observations to investigate this point, found that when he looked downwards during a spin he knew his position in space, but was conscious of forced movements. Thus, when spinning to the left (counter-clockwise), it would have been an effort to do anything to the right.

However well adapted a pilot may be to rotatory stimulation, he suffers from giddiness and frequently from nausea if he looks up at the top plane during a spin. Colonel Birley found that, on looking up, he 'lost himself entirely' and suffered from acute vertigo. 'I could not have done anything, and should have been incapable of all effort.' He was not, however, nauseated. Looking at the wing-tip is said by some to be even worse, and to be spun facing the tail is worst of all.

The account given by Captain H. is most instructive. During a spin he always looks straight forward at his instruments, and never lets his eyes wander outside the fusilage. On emerging from a spin he does not know where he is, but 'pulls out by rule'. He then finds that the 'horizon is crooked'. 'Something inside me would make me fly the machine one wing down.' During one set of experiments he looked up obliquely to the

left in order to count the turns by means of the sun, and was extremely uncomfortable. When he came out of the spin he was reading his instruments with great difficulty. 'I forced myself to do it; but it was a jolly big effort.' After coming out he stalled the machine badly and then dived; this was an unintentional manœuvre due to his state of mental confusion. He did not suffer from giddiness, but after it was all over felt sick. 'I felt bunged up and was unable to think clearly; I couldn't reason.' He is so familiar with this state that he has learnt by heart and repeats to himself exactly what he has to do, 'so that I mayn't have to think it out at the time'.

During these same experiments he carried out several more spins and nearly vomited. 'I felt something, under my lower jaw-bone, was being drawn upwards, and whenever I have felt this before I have always been sick. In this case I put my head over the side; but the drawing-up feeling below the tongue grew less and finally disappeared. I was not sick, though the feeling of nausea persisted for some time after I landed. I was very sick during the following night.'

Major V. B., a highly expert pilot, had the following experience whilst carrying out some experiments for the Technical Department. After an unsuccessful attempt to discover the smallest horizontal banked circle in which a Sopwith triplane could be spun, he got the machine into a turn at 3,000 feet. On starting the second turn 'the sky appeared to go grey'. 'A mist gradually arose like going under an anaesthetic,' and he 'fainted'. It was not an unpleasant sensation. When he came to himself, he was flying over a village about a mile away from the place of the experiment. The unconsciousness must have lasted about 20 seconds. During the first turn g reached 4·5, during the second 4·6. The turn was of about 140 feet at a speed of 114 miles an hour.

This pilot found experimentally that, whenever the acceleration (g) was pushed up to a high figure, he experienced the characteristic darkening of the sky which was preliminary to fainting. On another occasion he was looping and diving a D.H. 4 during a mock fight, when these preliminary sensations reappeared. He realized his danger and struggled against losing consciousness. First came a feeling of pressure in the head; then a mist gradually approached and spots rose before his eyes. He felt faint, chiefly at the bottom of the loop, and was quite conscious at the top. Then 'daylight' returned during the dive, and he came down about three minutes later feeling perfectly well. This preliminary sensation is distinctly pleasant, but is associated with inability to make an effort; 'it requires a definite struggle to right the machine.'

(c) *The Human Element in Aeroplane Accidents.*

(i) *The Physiological Effects of disturbances of Equilibrium.* Normal human beings differ profoundly both in the intensity and in the character of their reactions to changes in equilibrium. Rotation in a chair, with the head erect and the eyes closed, is

usually followed by a tendency to deviate, and, on opening the eyes, surrounding objects seem to be moving in a direction opposed to that of the rotation. Thus, if a normal man has been turned clockwise, the apparent rotation of the room goes counter-clockwise, and the deviation takes a form determined by the position of the head during the turning. The average human being in perfect health rapidly overcomes these after-responses, and recovers his stability in a few seconds; moreover, he does not suffer from nausea or other abnormal sensations.

Such a patient as No. 3 (p. 19) was classed as abnormal, although he was in perfect physical health, because he invariably became giddy and was incapacitated by stimuli to which the majority of men could adapt themselves without difficulty. Such a classification is, however, purely arbitrary. Aeroplanes are so constructed that they can be flown by any healthy young man without producing vertigo or other disturbing symptoms; it would be possible to build 'freak' machines that required the talents and training of an acrobat to resist the giddying effect of their evolutions. Thus the criterion by which we judge whether an aviator is normal or abnormal depends ultimately upon whether he can adapt himself to those disturbances of equilibrium to which he is commonly exposed in the air.

Giddiness and forced movements are not uniformly proportional to the rotatory stimuli acting on the body. For exactly the same physical forces, which produce intense vertigo when looking upwards, may be without effect if the head is held in the usual position and the gaze is directed horizontally forwards. This can be shown by the following experiment. Whilst standing in the normal position, rotate the head rapidly from side to side; most persons experience little or no vertigo. But, if the eyes are directed to the ceiling and similar lateral movements are carried out, the majority of healthy people become giddy and rock on their feet. The physical forces are the same in both cases: but in the former they are exerted with the head in its usual posture, whilst in the latter the position is one to which we are not habitually accustomed. In the same way even a normal pilot can be made profoundly uncomfortable by looking upwards during a spin.

An even more insidious danger is the mental state which accompanies the physiological changes induced by rapid rotation. Colonel Birley found that, when he was spun counter-clockwise with his eyes fixed on a spot directly below him, it became difficult 'to do anything' to the right; if he looked upwards during the spin, he became incapable of all voluntary exertion. Sydney Scott came to the conclusion that some persons do not come out of a spin because they cannot realize that they must make a definite mental effort to pull the machine into a state of normal stability. Several pilots of experience bore out this suggestion. One of them said that in putting on the rudder to stop a spin he is reluctant to substitute 'a state of strain for a state of ease'; his tendency is to continue spinning too long. Another spoke of this condition as a 'sort of mental coasting'. Major V. B. was particularly explicit on this point; he has been

throughout so conscious of the danger of this pleasant mental state that, as soon as he feels it beginning, he cuts short the manœuvre.

So long as the machine is one which will come out of a spin when the controls are released, the pilot may become unconscious and yet no accident happen. But if a definite act is required to terminate the rotation, as on a Camel, it is obvious that the machine may spin into the ground because the pilot has been rendered incapable of the necessary voluntary effort. The more rapid the turns the more likely is it that this condition will be induced; and it is not improbably responsible for some of the accidents on very delicate scout machines such as Camels. Where, however, the pilot is seen to fall into a spin, to come out again and to spin into the ground in the opposite direction, it is possible that forced movements are responsible for the reversal of rotation after the first recovery.

(ii) *Regression.* Flying, like the ability to play games, is definitely acquired and maintained by practice, and the measure we apply to all such human activities is the extent of functional efficiency. Now all action is based upon reflexes, which are controlled by the activity of higher centres; these in turn are dominated from above, and the active life of the central nervous system is comprised in a hierarchy of functions. Finally, consciousness stands as commander-in-chief over a vast number of subordinate activities, each of which is itself in authority over some more lowly mechanism.

To refer everything to the central authority would be a slow and laborious method of action; power is therefore delegated to the various departments to act without reference to the central administration. The lines of action are, however, laid down from above; we acquire a new aptitude by conscious effort excited through lower motor and sensory mechanisms. When learning to play the piano the fingers are laid consciously on each key and a complicated apparatus is thrown into action which strikes the note. Soon, with practice, the fingers move automatically and consciousness is occupied in reading the musical symbols on the printed page. Finally, with increased facility even this becomes almost automatic; the expert musician scarcely sees the page, for the nature of the musical phrase suggests to him the sequence of the accompanying harmonies.

First, the mechanical apparatus must function perfectly; the strings must be in tune and the levers which move the hammers perfectly adjusted. The fingers must learn to strike the right keys and the two hands be co-ordinated and controlled. This after long practice becomes automatic. At the same time there must be perfect comprehension of the musical notation and strict combination of mind and hand. Finally, in the supreme state of functional aptitude, the printed page suggests a musical thought which is automatically manifested as a series of sounds.

In the same way the aviator slowly acquires increasing facility in manipulating a complicated apparatus. At first he laboriously flies by rule and has to memorize the steps by which each evolution is induced. As he becomes more expert he has but to desire

some movement and it occurs; for a good pilot flies automatically and not by formula. He corrects, and may almost be said to anticipate, 'bumps' by instinct.

This power over the machine has been gained by conscious effort, during which a series of functional adaptations become engulfed in the automatic activities of the central nervous system, as soon as they are acquired. Movements laboriously learnt become unconscious reactions. But this supreme control can only be maintained by constant exercise and perfect physiological efficiency. Any influence which tends to diminish the power of concentration or to disturb the acquired mastery over sensory and motor impulses will lower ability to fly. The pilot becomes aware that he has lost his certainty in landing or is inclined to make flat turns. He does not know why he is no longer master of his actions, and complains 'I do everything that used to make me land perfectly, but it only comes off once in three or four times'. Like a golfer, he is 'off his game'. He does not know what is wrong because, when perfect facility has been acquired, the act has become automatic.

It is a well-known physiological law that when the control by a higher centre over some lower activity is diminished the character of the reaction tends to take on a more primitive form. The lower centres released from control are free to exercise their activities unchecked. For example, in hemiplegia the paralysed arm is frequently in a condition of great rigidity. This is due to the loss of that control usually exercised by the cortex of the brain over the lower motor centres. In the same way removal of the dominant influence of the sensory cortex on the afferent activities of the optic thalamus causes the paralysed half of the body to become over-sensitive to all painful or pleasurable stimulation. This phenomenon is usually spoken of as the 'release' of lower centres.

Here one crude structural centre of later development dominates another with more primitive reactions. But a similar law governs the inter-relation of a series of functions, each of which has been in turn acquired by practice and conscious effort. On the piano the fingers must first be taught to strike the right keys: then the two hands must work in a co-ordinate manner. As each new facility is gained it becomes automatic and disappears from the focus of consciousness. But with loss of control, movements once performed without thought require an effort of attention, and both mind and hand make apparently inexplicable mistakes. If the error takes the form of some fault previously overcome by practice we speak of 'regression'. The sufferer has fallen back on some previous method of reaction.

This unexpected return to some previous habit is one of the commonest human errors in all mechanical activities. Innumerable instances can be gathered from games and from daily life. A man who has driven a motor-car with the levers to the left, and then becomes habituated to the more ordinary form with a right-hand brake, may find in an emergency that he is groping to the left. A golf-player who is 'off his game' finds that he has fallen back to some long-conquered fault. This might be called

mechanical regression; some action, which has been acquired in perfection and has become automatic, may be disturbed by failure of control and is consequently carried out in an inferior manner.

But regression may be manifested not only in a motor act, but also on the sensory side. A tendency to giddiness in the air may be overcome with the acquisition of aptitude to fly. The physical stresses and strains to which the pilot is exposed remain the same, but the afferent impulses they cause are integrated and controlled. If, however, the general physiological resistance of the nervous system is lowered, or the power of mental concentration diminished, sensations of unusual intensity may be aroused accompanied by giddiness and nausea. A pilot may regress to the form of reaction which troubled him when he first went into the air.

The war has furnished frequent examples of the mental aspects of regression. A soldier is sent home suffering from the stress of fighting; he complains of horrible dreams, which take the most diverse and individual forms. Night after night he wakes in fear. But after suitable treatment these terrors cease and his dreams are occupied with the petty worries of the day. Suddenly all the old horrors return in consequence of some great sorrow or anxiety, such as the death of his wife or illness of a child. He has regressed to a previous psychical reaction in consequence of diminished mental control.

Not uncommonly all aspects of regression are manifested in the same patient. A pilot finds he is no longer flying with certainty; he is obliged to think of what he is doing instead of carrying out the manœuvre automatically. This frightens him, and the fear causes widespread regression. He may become giddy or faint in the air, and will probably 'crash' unexpectedly. He complains of frequent nightmares; these may be concerned with horrible events, previously forgotten, which occurred when he served in the trenches. Or he may be haunted by visions of a machine falling in flames, the reappearance in symbolic form of an old fear on first flying over the lines.

Thus regression may lead to the loss of mechanical aptitude, to an abnormal reaction due to the uncontrolled activity of sensory impulses, or to the reappearance of some less efficient form of mental activity. It is of profound theoretical interest, and probably explains certain apparently incomprehensible accidents in the air.

E. General Summary.

1. Conscious stability and comfort in the air depend primarily on the normal response of three groups of sense-organs. These are the vestibular apparatus, the eyes, including the eye muscles, and the proprio-ceptive system with its endings in muscles, tendons, and joints.

But of all the afferent impulses evoked by the impact of physical forces on these various mechanisms, those underlying the projected aspects of sensation are alone of significance in the

present inquiry. We are interested in those impressions which lead to recognition of the position of the body in space.

2. Defective responses from any one of these afferent end-organs will disturb the sense of equilibrium in the air, and may lead to actions that affect the stability of the machine.

3. No candidate for service in the R.A.F. is accepted who shows gross abnormality of any one of these primary organs of sensation.

But in the course of flying a machine heavier than air, an otherwise healthy man may suffer from disorders of vestibular and visual sensibility due to temporary causes. He may not be able to regulate the tension in the middle ear with sufficient ease during a rapid descent and consequently becomes giddy; or his eye muscles may no longer function strictly in unison, and he may develop heterophoria with its resultant lack of visual orientation.

4. The tests at present in use by the Examining Boards for estimating the sense of balance may reveal the existence of some abnormal functional state, but are not otherwise an indication of ability to fly.

5. Standing on one foot with the eyes closed, walking a line heel to toe, and balancing a rod on a flat board, are not tests for 'Muscle Sense' or 'Vestibular Stability', in the class of young men from whom the pilots and candidates of the R.A.F. are drawn. Failure to pass these tests, however, indicates some want of afferent or efferent central control.

6. The presence of Tremor, especially of the hands, is of great importance; it points to some distinct defect in static control, and is definite evidence of disordered function.

7. In some men, otherwise normal, rotatory stimuli produce a more powerful reaction than in the majority of their fellows. They become giddy easily. As children they could not swing with pleasure, and some of them were habitually sick in the train. Such persons are not suffering from a pathological condition; their disability arises from the fact that sensory impulses from the semicircular canals are not checked and controlled to the same extent as in the majority of healthy individuals. Consequently, when exposed to disturbances of equilibrium to which a normal man would adapt himself unconsciously, they suffer from discomfort, become giddy, and may even vomit in the air.

8. Many who were giddy when first exposed to aerobatic evolutions, succeed in conquering this disability. They learn to fly with ease, and the acquisition of this new facility is associated with control of the primary afferent impulses from the auditory and visual apparatus.

Any condition which produces diminished control by the highest faculties over the activity of lower centres, is liable to lead to regression to the earlier mode of reaction.

9. All healthy men can be affected if the intensity of the rotatory stimulus is pushed up to a sufficiently high degree. This may occur either from the fact that the head is held in some unusual position, as for example when looking upwards during spinning, or the actual rotation may be mechanically increased in rapidity.

In some cases the reaction to such excessive stimulation takes the form of giddiness with or without nausea; in others it is associated with that withdrawal of consciousness known as 'fainting'. When a man is made giddy he is in a state of confusion with regard to his relations in space. In the preliminary stages of 'fainting' his horizon is restricted and the world around disappears. Both states, pushed to the limits of endurance, end in unconsciousness.

10. Complete adaptation to disturbances of equilibrium in the air is associated with automatic control of the afferent and efferent activities of lower levels of the central nervous system. A perfect pilot desires that his machine shall behave in a certain manner, and the evolution occurs, exactly as an instrument in the hands of a skilled musician emits the appropriate sounds as soon as he becomes conscious of some harmonious phrase.

11. This acquired facility can be disturbed by any condition which leads to diminished control. Just as a golfer can be 'put off his game' by some physiological or mental state, so the aviator may lose his capacity to fly in consequence of conditions that vary so widely as a gastro-intestinal attack or domestic anxiety. Exhaustion, insomnia, the distress of war flying, anxiety or fear, can all lead to defective afferent and efferent control. Efficient automatic response is no longer possible, and the pilot begins to fly badly and to make bad landings.

12. But this loss of control over the activity of lower levels may be manifested not only in bad management of the machine, but in some dangerous reaction such as 'giddiness' or 'fainting' in the air.

13. Should this abnormal reaction assume the form of giddiness and nausea, it will be found in most cases that the patient has suffered from some discomfort when he first went into the air. Inquiry will usually reveal that he was unable to swing with pleasure as a child, and that he tended to be sick when travelling by train.

On the other hand, all the five pilots who came under my care for 'fainting', enjoyed their first flight and could swing with pleasure.

In the first group rotatory impulses, when released from control, manifested their influence in giddiness, usually accompanied by nausea. Amongst those who 'fainted' in the air the resistance to vertigenous impressions was high and no excessive giddiness resulted; but they induced the effects of shock with undue facility.

12.—MENTAL APTITUDE FOR AVIATION

BY

W. H. R. Rivers, M.D., F.R.S., and Squadron-Leader T. S. Rippon, O.B.E., R.A.F.M.S.

(In collaboration with the late Lieut. E. G. Manuel, R.A.F.)

(*Received October* 1918.)

The chief object of our investigation was to discover whether it is possible to detect a mental aptitude for aviation by means of a conversation.

With this aim two of us (E. G. M. and W. H. R. R.), independently of one another, conversed with a number of pupils under instruction at Hendon, and endeavoured to form an estimate of their capacity for flying.

All those examined were put through the Domino Test by another of us (T. S. R.), who estimated the mental aptitude on the ground of this test and their general demeanour. The conversations took as a starting-point the answers to the following series of questions :

Name.
Age.
Previous service and regiment, if any.
Married or single.
Occupation in civil life.
What school did you go to ?
How many boys were there ?
In what form or class were you ?
What games did you play ?
Did you play for your school in any game or games ?
What was your age on leaving ?
Do you ride a horse, hunt, sail a yacht ?
Do you motor ?
What is your favourite amusement ?
Who is your favourite author ?
Who is your favourite poet ?
Do you take alcohol ?
Have you ever recited or made a speech in public ?
Do you mind solitude ?
What is your attitude to flying ?

These questions were designed to give an outline of the pupil's history and some of his interests before joining the R.A.F. The information so obtained was taken into account in estimating the

aptitude for flying, but it was of less weight than the general impression made by the pupils, and information which would never have been given in the answers to a *questionnaire*. It is important to note that the conversations were private, thus getting rid of any constraint due to the presence of onlookers. We endeavoured not only to say whether in our opinion pupils would or would not succeed as airmen, but to grade them to different kinds of flying. We grouped them in four classes. In Class A we placed those whom we thought would make fighting scouts ; in Class B those who would be successful in other kinds of flying ; in Class C we placed those we expected to succeed in graduating, but who would not become good pilots, and Class D was for those we expected to be total failures.

For several reasons the investigation was unsatisfactory, and little weight can be laid on any results which have been obtained.

Our work forms little more than a preliminary study adopted to discover how an investigation of this kind should be carried out in future.

Some of the defects of the work are due to the unfortunate death of Lieut. Manuel in a flying accident. When this occurred we had only examined 37 pupils. Of these two had already been found to be unsuited for flying when they came to us for examination, and two others had had crashes, introducing a complication which made their exclusion necessary.

Our work is therefore based on the examination of the wholly insufficient number of 33 subjects.

A second defect of our work is that we had not at the outset made up our minds clearly concerning the nature of the aptitude we were studying. Our attention was directed chiefly to the capacity for flying, and we paid too little attention to endurance and capacity for fighting, which are quite as important as the capacity for flying in relation to the present needs of the Air Force, and, as already mentioned, our grading was based chiefly on our opinion whether the pupil would make a good scout pilot. These pupils formed our A Class, and we put in the second class men whose powers of endurance and capacity for fighting would make them quite as efficient members of the Air Force as many of those we put in the first class. Our attitude was changing even during the short investigation. If it had not been for Lieut. Manuel's death we should have certainly begun again with a more clearly formulated system of grading.

A third and very serious defect is that all the pupils had already had some experience of flying when they came to us. This greatly diminishes the value of our work as a guide to the use of conversations as the means of detecting aptitude in candidates for admission to the R.A.F. We tried to base our opinion on evidence independent of flying experience, but inquiries into the attitude towards flying are essential in any such work as we were attempting, and in these inquiries it was impossible not to be influenced by references to actual flying experience. These, indeed, would play a part even if we had rigorously excluded all reference to flying.

Finally, our work is incomplete, owing to our failure to obtain any record of the progress of many of the candidates, and even where we have received reports, these in many cases give too

little information to allow any satisfactory comparison with our estimates.

Reports have been received concerning 21 of the 37 pupils examined. Of these progress has been reported as ' very satisfactory ' in four cases ; ' satisfactory ' in six ; ' average ' one. In two cases pupils have been found unsatisfactory as scouts, and had been recommended for heavier machines, and two other cases were reported ' unsatisfactory ' without qualification. In three cases the report is that the pupil has done no flying and in one that he is ill. Three of the small number we examined have been killed : in two of these cases no information is given concerning progress before the fatal accident, but in the third case the pupil had made very satisfactory progress in Camels when he was killed, owing to a wing breaking off his machine. In the following table, which gives the estimates of the three examiners, this pupil is therefore entered under the heading ' very satisfactory '.

	E.G.M.	*W.H.R.R.*	*T.S.R.*	*Domino Test.*
Very satisfactory.				
C.B. 22	A	B	B	10–67
S.K.F. 21	A	B	B	10–67
A.B.R. 18	A?	A	A	10–57
P.G.W. 24 (killed)	A	B	B	9–63
Satisfactory.				
S.G.B. 23	B	B	B	11–62
R.E.N.	A	A	B	11–93
A.B.S. 19	A	B	B	10–72
W.R.W. 21	B	B	B	10–66
G.H.W. 19	B	A	B	11–61
W.C.G. 23.	A	B	D	10–80
Average.				
L.B.J. 19	A	B	A	9–54
Unsatisfactory scouts. Recommended heavy seaters.				
F.T.C. 23	B	A	A	12–72
G.E.P. 21	B	B	A	8–54
Unsatisfactory.				
A.B.R. 19	B	B	A	10–68
W.G.W. 28	B	C	B	10–62
No flying.				
H.T.B. 27.	—	B	B	10–72
J.C.B.	B	B	B	11–83
G.H.S.	A	A	B	11–84

Ill. 1.

	E.G.M.	*W.H.R.R.*	*T.S.R.*	*Domino Test.*
E.V.W. 19 . . .	B	B	A	9–49
	Killed.			
F.B. 22	B	B	B	10–58
J.C.D. 18	A	A	B	10–63

The most striking feature of these results is that Lieut. Manuel was very definitely more successful in estimating the aptitude for flying than either of his medical colleagues. Only in one case, that of L. B. J., was he definitely wrong.

The numbers are too few to allow any estimate of the value of the Domino Test, but the general impression given by the results is that taken alone it is of no great value. Of the two candidates who did it in the shortest time, the progress of one was unsatisfactory, and the other had only an average record. Of the four candidates who were found 'very satisfactory' only one did the Domino Test in less than 60 seconds.

A much larger number of examinations, however, should be made before any decision concerning this value of the test should be reached.

Though our work is based on too small a number of examinations and has many defects of method, it has been useful in pointing to the methods upon which such an investigation should be carried out in future. We therefore make the following suggestions :

(*a*) An investigation designed to discover whether it is possible to detect mental aptitude for the work of the R.A.F. should be carried out on pupils before their admission to a flying school, and most appropriately at the school to which they go immediately after admission to the Force.

(*b*) It is not enough to grade simply into classes according to general aptitude, but it is necessary to distinguish between such features as manipulative ability, fighting capacity, endurance, and intelligence.

The following scheme illustrates the nature of the grading which is now being used at the 29th Training Squadron :

Ability.	*A.*	*B.*	*C.*	*Remarks.*
Manipulation .				
Fighting . .				
Endurance . .				
Intelligence . .				

(*c*) Our work suggests that pilots who have the necessary interest in the subject will form the most suitable persons to act as examiners.

(*d*) The fatiguing nature of such examinations makes it quite impossible that they can form part of the general tests

for admission to the R.A.F. The existence of mental aptitude for flying is probably most important in borderline cases, among whom the presence of definite aptitude might be allowed to counterbalance a degree of anatomical or physiological defect which would otherwise lead to rejection. For this purpose special examiners might interview selected cases, or this inquiry might form part of the work of the assessors. In general, however, inquiries into mental aptitude would come most appropriately at an early stage in training, and should be used as one of the means of excluding unsuitable candidates who have succeeded in obtaining admission to the R.A.F. on the ground of physical and physiological suitability.

APPENDIX ON 'WIND-UP'

BY

W. H. R. Rivers, M.D., F.R.S.

The expression 'wind-up' was probably used originally for any state of mingled excitement and apprehension called into being by an unusual occurrence, and especially the prospect or actual presence of danger. It has, however, gradually come to be used in the army as a means of expressing fear in one or other of its forms, an expression by means of which a soldier will talk about fear without explicitly acknowledging the presence of this emotion. I propose here to use the word as a definite expression for fear.

Fear is the emotional or affective aspect of the instinctive process called into activity by danger. It is the modification of consciousness which accompanies certain instinctive forms of action in response to danger, and especially the response by flight. It is especially intense when there is interference with this or any other form of reaction to danger.

It is only in Man that we are able to study by means of introspection the various forms of fear. The first distinction to be made is between the emotional state or states accompanying the actual presence of danger and the various forms of fear which arise when there is only the prospect of danger, while in pathological states a large group of fears or states allied to fear occur independent of either actual or prospective danger.

Reaction to actual danger. The most frequent reaction to danger in Man is one of heightened capacity for the activities by which the danger may be met without any trace of the fear which, if present, would inevitably interfere with this capacity. A man in the presence of danger will carry out with the utmost coolness, and often with a degree of skill surpassing that he usually shows, the measures necessary for the aversion of the danger or his escape from it. In such a case there is complete suppression of the emotion of fear which the danger might be expected to produce, and this suppression is nearly always accompanied by suppression of pain, so that an injury derived from the dangerous object or from any other source is not perceived.

A second mode of reaction is the assumption of an aggressive attitude towards the source of danger with the accompaniment of the affective state of anger. In this case there is not a simple suppression of fear, but its place is taken by another emotion belonging to the instinct of aggression. If these lines of action fail, if the serviceable activity which would lead to escape from the danger is interfered with or becomes impossible to carry out, or if the aggressive reaction does not succeed, fear supervenes as an accompaniment either of flight or of the collapse which is apt to occur when the more normal and serviceable reactions fail. In some cases, however, the suppression of fear is so well established that this emotion remains completely absent even when the danger is so insistent and unavoidable that death or violent injury is inevitable. Thus, the emotion

of fear may be completely absent during the fall and crash of an aeroplane in which death seems certain, its place being taken by an interest such as might be taken by the mere witness of a spectacle, or by some apparently trivial line of thought. It is when some line of action is still possible, but this action is recognized to be fruitless and in vain, that fear, often in the acute form we call terror, is likely to supervene.

Reactions to prospective danger. The state most commonly produced by prospective danger is one of that degree of fear which we call apprehension. This may be so intense as to become indistinguishable from the fear which accompanies the active presence of danger, but it is more usually a vague discomfort, with minor degrees of the tremor and muscular weakness which accompany fear.

This state of apprehension may occur, often in a relatively intense form, in men who become perfectly cool and collected as soon as the danger becomes actual, when the state of apprehension completely disappears so that there is no interference with the activity by which the danger may be averted. The apprehension preceding the occurrence of danger is of exactly the same order as stage-fright or the fright preceding any other public performance, and just as the best actors and orators are liable to stage-fright, so may those who show the utmost coolness and bravery in the actual presence of danger be liable to apprehensions while the danger is still only in prospect.

In other cases the apprehensions at the prospect of danger are so acute and so accompanied by physical manifestations which make appropriate action impossible that the actual occurrence of danger only serves to bring about complete collapse.

Pathological fears. Fear is a very frequent accompaniment of pathological states, and many of its more extreme forms only occur in adult Man as part of such states.

The most frequent form in which such intense fears arise is the nightmare or the night-terror of the half-waking state. These are especially characteristic of childhood, but they may occur in adult life in those who seem otherwise healthy, while they have recently become familiar as the most prominent symptom of states of anxiety arising out of the war.

Similar intense fears may occur in the first stage or following the administration of an anaesthetic, or attacks of terror may occur in the waking state as part of an anxiety-neurosis.

Another pathological form taken by fear is shown by the various phobias, in each of which some special stimulus may arouse fear in one who otherwise may not know what fear means. The stimulus which thus arouses fear, often in a very intense form, may be one which in other persons not only wholly fails to arouse this emotion, but may be a source of definite pleasure. Thus, a man who does not know fear in the presence of actual danger to life or limb, may suffer from acute fear in the presence of a cat or a harmless snake, or an airman who is only stimulated by the utmost danger of aerial warfare may suffer from acute apprehension in a lift or on a ladder only a few feet from the ground. These highly specialized fears also occur in relation to definite sources of danger; thus, one who is undisturbed by most of the dangerous situations of warfare may have some special fear, whether of searchlights, sniping, or some special kind of shell.

Still another form of fear is the more or less persistent state of anxiety which forms so prominent a feature of the functional nervous disorders arising out of warfare that it has been adopted in the nomenclature of one of the most frequent forms taken by these disorders. In the healthy person anxiety is a state which comes into existence in consequence of some prospective misfortune or danger, but in morbid conditions it shows itself in the form of more or less continuous apprehension colouring the whole mental life, so that even the most ordinary occurrences are seen in the blackest light as sources of trouble or danger.

Suppression and repression in relation to fear. In the form of reaction to danger which seems to be characteristic of the normal healthy man, there is a complete absence of the emotion of fear. No effort is needed to keep fear out of the mind, for it shows no tendency to appear in consciousness, and yet fear in the presence of danger is so necessary a part of the mental equipment of animals and is so frequently manifested in childhood that we can confidently assume

this emotion to be potentially present but in a state of suppression. This assumption is supported by several lines of evidence. A man who when exposed to danger experiences no trace of fear and behaves with the utmost coolness and bravery may yet suffer subsequently from acute fear in his dreams. If, as there is much reason to believe, suppressed affective states find expression in dreams owing to the weakening of control normally exerted in the waking state, the occurrence of fear in dreams following a dangerous experience would be a natural consequence of its ordinary existence in a state of suppression.

Still more important and conclusive is the occurrence of fear as the result of shock or long-continued strain and fatigue which lower the efficiency of the higher controlling levels of mental activity. Thus, one of the earliest signs of the strain of warfare is the occurrence of apprehensions in one who till then has passed through the dangers of warfare without fear. The occurrence of fear either manifestly or in the form of vague apprehensions when shock or strain has lowered efficiency is naturally explained if the fear has been there throughout, but in so complete a state of suppression that it never passed the threshold of consciousness. As soon as fear or apprehension begin to show themselves in consciousness, a new process comes into action. The fear, no longer held unconsciously in check, has now to be consciously repressed. One who has flown or fought, perhaps for many months, without knowing fear, finds himself the subject of apprehensions which he regards with shame and strives to banish from his mind. A short rest at such a time, by allowing the unconscious controlling process again to take the upper hand, will often bring about the disappearance of the apprehensions so that danger can again be faced with equanimity and without the necessity for conscious repression. Or the lowered efficiency of the controlling forces may be temporary and the recuperative power of the sufferer may be so great that recovery of the normal state of suppression may come about, so that conscious repression again becomes unnecessary. More frequently, however, the conscious repression of fears or apprehensions only adds to the strain and fatigue which has led to the failure of suppression. The fears become stronger and call for still stronger efforts of repression. Through the vicious circle thus set up there is produced a state of persistent anxiety in which even ordinary incidents of life, incidents wholly devoid of danger, come to be viewed with apprehension. The fears which are repressed with apparent success during the day find expression in an accentuated form at night, when the control exerted by day is removed in sleep or weakened in the state preceding or following sleep. The interference with rest so produced only serves to increase the state of strain and fatigue to which the nightmares or disturbing night-thoughts are primarily due, while disturbances of digestion or circulation due to the anxiety may react on and accentuate the state to which they are primarily due. Finally, some shock or additional strain, a slight accident which a few months before would only have raised a laugh, a misunderstanding with a superior officer or some domestic trouble, will bring about a crisis and reduce the soldier to a state in which he becomes wholly unfit for any kind of duty. The morbid state which most frequently supervenes is that known as anxiety-neurosis, which is only an exaggeration of the morbid state of anxiety which preceded his definite breakdown. In other cases, the trouble may find expression in some mimetic disability usually known as hysteria, while in those of psychopathic disposition, there may be complete mental collapse, or the unbearable situation may be solved by the occurrence of those false rationalizations which we call delusions.

The special feature of practical importance in the foregoing statement of the various forms taken by the emotion of fear is that the occurrence of this emotion may be a symptom, often the earliest symptom, of a state of fatigue and strain. Owing to the way in which the society to which we belong, and especially those whose business it is to fight, look upon fear, its occurrence, especially without adequate cause, arouses other emotions, and especially that of shame, which greatly enhance the strain to which the fear is primarily due.

Treatment. It is evident that the state so produced is one which gives ample scope for treatment, both preventive and curative. There is no department of medicine in which a medical officer can gain results so definite as in the treatment of the early stages of the anxiety-neurosis of warfare. The earlier he can act the better, for the longer the state of anxiety is allowed to last, the greater

the conscious repression which becomes necessary, the longer is the period of rest which is required to enable the process of suppression to become again effective. Moreover, the occurrence of disturbances of circulation, of digestion and of other organic functions may produce complications which greatly prolong the process of recovery. Nowhere is the adage more appropriate that a stitch in time saves nine.

A medical officer can only hope to succeed if he is on such terms with those under his care that they are ready to give him their full confidence, for owing to the general sentiment regarding fear, it is only with the greatest reluctance that its presence is acknowledged. It is here that the expression 'wind-up' has its peculiar utility in that it enables one in whom strain is producing apprehensions to refer, half seriously, half humorously, to his trouble. The first step in the treatment is to assure the patient that there is no cause for shame, that the fear he experiences is a well-recognized symptom of strain and is due to the temporary failure of the mechanism by which in the healthy and normal man fear is kept under adequate control. If sleep is already disturbed by dreams, a second line of treatment will be to induce the sufferer to give up the process of voluntary repression to which, in the vast majority of cases, these dreams are due. Having by this process of education put the patient on the road to recovery, a short rest followed perhaps by a period of limited duty, will usually restore him to his normal level of efficiency. To send him for a holiday without the necessary process of education and reassurance is open to the serious risk that he will only continue during the holiday to repress or brood over his painful thoughts and feelings, with the result that the state of anxiety is accentuated and becomes a fixed habit.

Finally, it must be pointed out that this line of treatment only holds good for those in whom the occurrence of fear is clearly the result of shock or strain. Those who are naturally apprehensive require a different line of treatment. Their case is far more difficult and less hopeful than that in which fear is secondary to strain or shock, but much can be done with them by sympathetic encouragement in fighting their disability, and when possible, by gradually introducing them to the conditions which rouse their apprehensions. There is reason to believe that in some cases such apprehensions are the definite sequel to some emotional shock in childhood or youth which has set up faulty trends in feeling and behaviour. In such cases, a thorough and sympathetic discussion of the history of their fears may be of great service and may at least allow the medical officer to recognize how far the state is capable of amendment, whether there is a reasonable hope that the patient may acquire that state of suppression of fear which in his more fortunate comrades has come into existence in childhood.

13.—'REACTION TIME' TESTS CARRIED OUT ON FLIGHT CADETS, WITH DESCRIPTION OF APPARATUS

BY

The late L. E. Stamm, M.D. (late Lieutenant R.A.F.)

(*Received November* 10, 1918.)

For the past year or so the value of employing 'reaction time' tests in the selection of candidates for aviation in the Air Service has been under consideration. In France a scheme of simple reaction time tests has been adopted, by which a normal neuro-muscular response to the different senses is ensured. In America the simple reaction time tests have been rejected as too automatic and of no value for the purpose in hand, and more complex tests have been adopted, in which conscious mental processes of discrimination are involved.

On *a priori* grounds it appeared to me that simple reaction time tests could be of little practical value for the Air Service, because in healthy young men with normal senses and muscles, the co-ordination between these and therefore the 'reaction time' is sufficiently quick and accurate for all practical purposes, and in actual experience I have obtained some comparatively slow reactions from very capable individuals and very quick responses from some of poor mentality. In candidates for the Air Service I do not think these simple reaction time tests would eliminate 1 per cent. of undesirables.

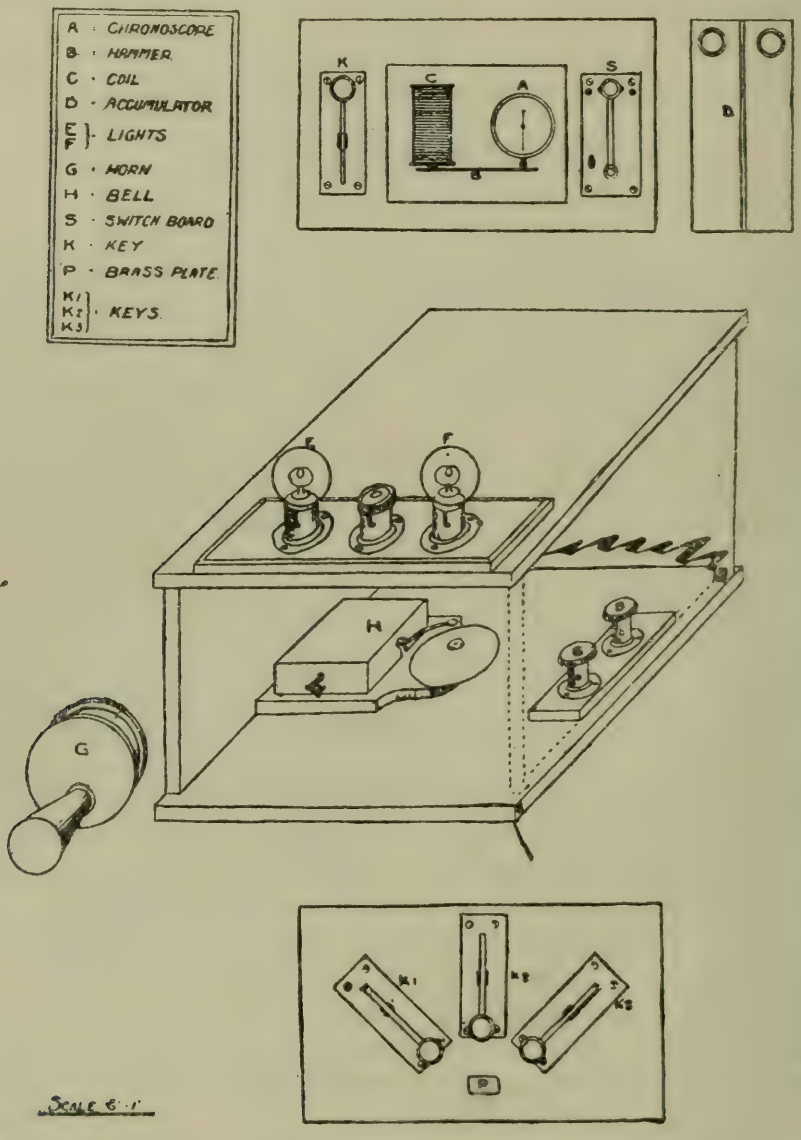
What one requires to test is the ability of the candidate to respond quickly and accurately under conditions in which the higher *conscious* mental functions of discrimination, decision, and judgement are involved. For this purpose one has a number of different signals, requiring different responses, the candidate not knowing which signal to expect. This necessitates the intervention of the conscious mind between the perception of the signal and the motor response, and consequently the reaction time is lengthened. The mental ability is tested by the rapidity and accuracy of response in proportion to the complexity of the problem presented to the candidate.

Many forms of apparatus have been devised, but as the interval of time is only a fraction of a second, and variations are measured in 1/100ths of a second, some difficulty has been found in obtaining accurate and reliable apparatus that is also sufficiently simple for practical purposes.

With the help of Major Burgess, engineer, R.A.F., I have devised an apparatus which is comparatively simple, and which in practice has proved of value in testing candidates for the Air Service. A diagram of the apparatus is shown below.

Reaction Time Apparatus

It consists of a chronoscope A, which registers to 1/100 second, and is worked electrically by a hammer B, from a coil C, and accumulators D. There are four signals, two differently coloured lights E and F, an electric horn G, and an electric bell H. The operator has a switchboard S, by which any of the signals is put in the current,



and the signal is given by touching the key K. The candidate sits opposite the operator and places his right-hand first finger on the brass plate P, which is equidistant from the three keys K_1, K_2, K_3. The end keys, K_1 and K_3, are for the corresponding lights, and the middle key, K_2, is for the horn or the bell. It was not considered desirable to complicate things to the extent of four keys.

When the operator presses his key K one of the signals is given, and at the same time the chronoscope starts. The candidate responds by touching the appropriate key, K_1, K_2, or K_3, which stops the chronoscope, and the reaction time is read off in 1/100th seconds.

The method of examination is as follows: After a short pre-

liminary rehearsal to acquaint the candidate with the method of working the apparatus, the candidate is tested for the simple 'automatic reaction time' by one signal, which he knows beforehand. He is tested in this way by a series of each of the four signals. As already stated, I set no value on these tests, but they enable the candidate to get used to the apparatus. After testing for the simple reaction time with each separate signal the candidate is now told that he may get either one of the two lights, and he is to respond by touching the appropriate keys K_1 or K_2. Having done a series of ten with the two light signals, he is now given a series with three possible signals, the two lights and the horn, and finally a series of all four signals—two lights, horn, and bell—but for the bell for which he has previously used the middle key, K_2, he is now told to keep his finger on the brass plate. This involves mental inhibition. Each series shows an increase in the reaction time, owing to the increased complexity of the problem before the candidate. Recently I have used a further series of four signals, in which the keys previously used for the lights, each corresponding to the light on the same side, are now reversed. This further complicates the problem and causes a further increase in the reaction time.

The following are the averages of 150 cases, including pupils and instructors (good pilots), for the different tests in 1/100th seconds :

(1)	Reaction time to single light signal	28
(2)	,, ,, ,, sound ,, ...	26
(3)	,, ,, two lights	35
(4)	,, ,, ,, ,, and horn	38
(5)	,, ,, ,, ,, ,, two sound signals	44
(6)	,, ,, four signals with keys to lights crossed	48

Examination of 20 instructors (experienced pilots) in the same way yields, as would be expected, appreciably lower figures as averages for the 20 cases :

Simple visual reaction (one signal)	27
,, auditory ,, ,, ...	25
Two signal test (two lights)	32·4
Three ,, ,, (two lights and horn)	34·5
Four ,, ,, (two lights and two sound signals)	40·4
,, ,, ,, (with keys and lights crossed) ..	46

Taking the last two tests as the crucial tests of the mentality of the pupil, I have found a remarkable correspondence between the results and their capacities as flying pupils. Having tested a certain batch of pupils, I send the names to their instructors, and ask for their opinion as to their flying capacities and possibilities of making good pilots. Arranging them in the order of the results from the machine, as a general rule the top names have 'very good' or 'good' against them, the middle ones 'fair' or 'average', and the bottom ones 'poor' and probably 'turned down' or sent to 'heavier machines'.

Appended is a list of pupils examined, with the results of the test

and the criticisms of their instructors, from which the value of the test can in some measure be estimated.

I should point out that with my apparatus there is a muscular adjustment involved in the movement of the finger to the different keys. In my opinion this is a valuable factor as a further test of the nerve mechanism, but it necessarily prolongs the reaction time, so that even the simple reaction time with my apparatus is longer than the usual without such muscular adjustment. It is quite easy to dispense with this for simple reaction to one signal by placing the finger ready on the key.

The value of the tests lies in the fact that they involve considerable adjustment and co-ordination between the senses and the muscles, and at the same time call for a rapid and accurate working of the higher mental functions of conscious discrimination and decision, a complexity of conditions similar to that involved in piloting an aeroplane.

Moreover, it calls for a prolonged effort of attention, and the ability to sustain the effort is shown by the way in which a candidate succeeds in keeping up his rate of response to the end of the tests, or shows evidence of mental fatigue by sudden lengthening of the time intervals or by mistakes in the keys.

Over and above everything else there is the 'will' factor to be taken into account. This may act in at least two different ways. It may be negative. Then the results of the tests will not represent the best that the candidate can do. Or it may be very positive. It may be exercised to such an extent that the candidate exceeds for a time his normal power of reaction. In this case the excess of his efforts usually shows itself by mental fatigue setting in before the tests are finished, and he is unable to keep up the pace he has set for himself. All these variations are very useful to observe in forming an estimate of the candidate's mental constitution and capacity.

I would, in fact, claim for the apparatus that it not only gives figures of some value for estimating the mental capacity of a candidate for aviation, but that it affords a means of employing a candidate in a way which gives considerable opportunity of making mental notes in regard to his intelligence and personality, by the manner in which he tackles the machine. Some tumble to the game at once and show a remarkably quick adaptability, whereas others are equally slow in picking up the idea, but may warm up as one proceeds and give quite a good account of themselves in the final tests. Some are light in touch and accurate; others heavy and clumsy, so that they often miss the keys and almost break them when they touch them. I have myself been surprised in dealing with pilots personally known to me to observe how the machine seems to bring out and record their personality, and especially their flying personality. There appear to be two good but different types of mentality which react differently to the apparatus. In the one the reaction times are very quick, but in cutting down the time allowed for discrimination to the utmost in the final tests several mistakes may be made. In the other good type the reaction times are appreciably longer, perhaps even above the average, but the signals are responded to with

absolute accuracy. The flashy, crack scout pilot shows the first type of reaction, while the steady, careful pilot is characterized by the second type of reaction.

In addition to these mental qualities of quick discrimination and decision, for which I have attempted to test by this apparatus, there is also the factor of 'emotional stability', to which I have also given some attention. It will be found on comparing the results of my apparatus with the opinions of the instructors that this factor of 'emotional stability' is the chief disturbing element in the co-ordination of the two columns. Pupils giving good reaction time results may be marked 'poor' by the instructors because they are 'windy', and others showing long reaction times may be marked 'good' because they have 'guts'. Very often I have notes to the same effect.

I have found that the pulse-rate under ordinary medical examination is a very fair guide to detect the 'windy' type. People who manifest their uneasiness at coming before a doctor for examination by a rapid pulse, which does not settle down in spite of every effort to put them at their ease, prove by that fact that there is a lack of control of the nerve mechanism of the vital centres, that there is an instability and liability to serious disturbances by the emotion of fear, and they belong to the 'windy' type. This instability does not appear to be in the vital centres themselves, but in the higher nerve centres controlling them, because such individuals respond to the ordinary physical test in a normal manner. The manometer test, the exercise test, show a perfectly normal acting mechanism; it is the rapid pulse of emotional disturbance.

It appears to me that a combination of reaction time tests such as I have carried out, with some tests for emotional stability, such as the pulse-rate test and perhaps the galvanometer test, should offer very satisfactory results in the selection of candidates for aviation, in regard to their mental and temperamental suitability for the work.

FLIGHT GROUP No. I.

Name.	*4-signal test in 1/100 sec.*	*Remarks of M.O.*	*Instructor's Remarks.*
I.	31	Haphazard, W.7.	Good, lacks judgement.
II.	35	Very intelligent and quick.	Very good—now instructor.
III.	35·5	P. 120. Nervy but guts mentally.	Fair—nervy, drinks too much
IV.	36	Sturdy, sports, W.3.	Good but slow.
V.	38	Sturdy Canadian, horse-riding.	Good, quick, guts.
VI.	39·5	—	Average.
VII.	39·5	All there—guts.	Good—plenty of guts.
VIII.	39·5	Says upset by 3 instructors.	Poor, no guts, gets sick.
IX.	40	No nerve physique.	Average.
X.	41	—	Very good; steady.
XI.	42	—	Good.
XII.	42	Capable, good type.	Extra good.
XIII.	42	—	Average.
XIV.	42	Alert, good type.	Very good—now instructor.
XV.	42	—	Average, just started flying.
XVI.	43	Intelligent.	Good.
XVII.	43·5	Sports, average intelligence.	Fair—guts.
XVIII.	44	—	Average.
XIX.	44·5	Uptake slow.	Good but silly.

Name.	*4-signal test in 1/100 sec.*	*Remarks of M.O.*	*Instructor's Remarks.*
XX.	44·5	Lethargic.	Slow but solid.
XXI.	46	Dumpy.	Average, slow.
XXII.	46	Musical, nervy.	Fair.
XXIII.	46	Slow uptake, no sports, clerk.	Average.
XXIV.	47	Slow uptake, no sports, clerk.	Average, slow learning.
XXV.	47	Alert—sports.	Good.
XXVI.	47	Nervy.	Poor—windy.
XXVII.	47·5	Very anxious, W.4.	Average, persevering.
XXVIII.	48	—	Poor—sent to heavier machines.
XXIX.	49	Windy, paralysed by horn signal, bad type.	Poor—windy.
XXX.	50	—	Average, large flying experience.
XXXI.	50·5	Bank clerk, not robust.	Average.
XXXII.	50·5	Bright; guts.	Good—lots of guts.
XXXIII.	50·5	—	Poor. ? Windy. To be turned down.
XXXIV.	52	Slow but good type.	Slow, but will make good.
XXXV.	52	—	Fair.
XXXVI.	52	Sturdy—rough and ready.	Fair—solid—heavy-handed.
XXXVII.	52·5	Slow and dull.	Average to poor.
XXXVIII.	53	Nervy, sick on stunting.	Poor.
XXXIX.	55	Heavy—dull.	Poor—sent to heavier machines.
XL.	55·5	Dull and windy.	Poor—sent to heavier machines.
XLI.	57	—	Poor.
XLII.	75·5	Mentality very poor.	Hopeless.

FLIGHT GROUP No. II.

XLIII.	33	Journalist, M.A. Alert, no sport.	Good, but previous flying experience.
XLIV.	36·5	Average.	Good.
XLV.	37	Good type.	Very good.
XLVI.	37·5	—	Good—guts—keen.
XLVII.	39	Good type—Canadian.	Very good.
XLVIII.	40	Good type.	Good.
XLIX.	40	Average.	Good.
L.	40	Quick but bumptious and over-confident.	Good but objectionable.
LI.	40·5	—	Average.
LII.	40·5	Heavy Scotch type—guts.	Average, perseveres.
LIII.	41	Good type.	Very good—now instructor.
LIV.	41	—	Good.
LV.	41·5	Average.	Good, slow learning.
LVI.	41·5	Bright and intelligent; guts.	Very good and keen.
LVII.	41·5	Accountant, lethargic and heavy.	Heavy-handed; to be turned down.
LVIII.	41·5	Lack of muscular adjustment.	Poor and slow.
LIX.	42	Good type—sport.	Very good, steady and keen.
LX.	43	? Windy.	Good but windy.
LXI.	43	—	Average, rather slow.
LXII.	43	M.C.	Good but slow learning.
LXIII.	43·5	Heavy.	Good but slow learning.
LXIV.	44	—	Keen but lacks judgement, windy.
LXV.	44·5	Nervous temperament but not windy.	Average, keen, guts.
LXVI.	44·5	Sports, horse-riding.	Average, keen, guts.
LXVII.	45·5	—	Average but slow.

W. = mistakes in keys
M. = mentality. A. good. B. average. C. poor.
Where not stated mentality is average.

Name.	4-signal test in 1/100 sec.	Remarks of M.O.	Instructor's Remarks
LXVIII.	46	Good type.	Good.
LXIX.	46·5	Good type Canadian.	Good.
LXX.	47	Average.	Good and very keen.
LXXI.	47	—	Average to poor.
LXXII.	48	Little stamina, clerk.	Average.
LXXIII.	48	Average—no sports.	Average.
LXXIV.	48	M.C. slow.	Good guts—slow learning.
LXXV.	52·5	P. 96. Tremors, ? windy.	Average—windy.
LXXVI.	49·5	Average.	Slow—improving.
LXXVII.	50·5	Nervy.	Poor—windy.
LXXVIII.	51	Sports.	Poor but guts.
LXXIX.	53	M.C. not good type.	Very slow, to be sent to heavier machines.
LXXX.	53·5	—	Average
LXXXI.	54·4	M.C. heavy. W.5.	Hopeless, turned down.
LXXXII.	54·5	M.C. heavy.	Poor—very slow.
LXXXIII.	54·5	M.A. Bright. W.4.	Average.
LXXXIV.	56	Slow and dull.	Very poor—turned down.
LXXXV.	57	Windy—bad type.	Poor—heavy-handed.
LXXXVI.	58	Heavy—sports.	Average—guts.
LXXXVII.	58	M.C. but guts, drinks too much.	Average—guts.
LXXXVIII.	61	—	Poor.
LXXXIX.	66	Civil Service clerk.	Poor—slow.

FLIGHT GROUP No. III.

XC.	34	Alert—bright.	Very good.
XCI.	35	Average.	Average, not keen.
XCII.	38	Canadian, good type, sports.	Very good.
XCIII.	38	Good type.	Very good.
XCIV.	38	—	Average.
XCV.	39	M.A. Good type.	Very good—now instructor.
XCVI.	40	M.A. Rather slow in uptake.	Very good.
XCVII.	40	—	Poor—inattention.
XCVIII.	40	M.A. Alert.	Good.
XCIX.	42	Good type—sports.	Very good.
C.	42	Windy.	Intelligent but no guts.
CI.	42	Artistic. M.A.	Good.
CII.	43	—	Good.
CIII.	43	—	Good.
CIV.	43	—	Good.
CV.	43	—	Fair, lacks confidence.
CVI.	45	Good type—bright.	Good.
CVII.	45	No physique—windy.	Poor, windy and sick.
CVIII.	45	—	Average to good.
CIX.	45	Alert and bright, good physique.	Average to good.
CX.	46	Rough type.	Average to good.
CXI.	46	—	Good.
CXII.	46	Canadian, rather stolid—guts.	Good.
CXIII.	47	Canadian, sports, M.A.	Good but very slow.
CXIV.	47	Stolid but guts.	Good—guts.
CXV.	47	Russian, intelligent.	Average.
CXVI.	48	—	Average.
CXVII.	48	—	Average.
CXVIII.	48	Alert—guts.	Average.
CXIX.	50	M.B. Slow in uptake.	Average.
CXX.	50	—	Average.
CXXI.	50	Slow—stolid.	Good, guts.
CXXII.	50	—	Poor.
CXXIII.	52	Too talkative and plausible, windy.	Average—windy.

W. = mistakes in keys.
M. = mentality. A. good. B. average. C. poor.
Where not stated mentality is average.

Name.	*4-signal test in 1/100 sec.*	*Remarks of M.O.*	*Instructor's Remarks.*
CXXIV.	52	Bright and intelligent.	Average.
CXXV.	52	—	Average.
CXXVI.	52	—	Average.
CXXVII.	52	Good type Canadian, sports.	Average.
CXXVIII.	53	—	Average.
CXXIX.	54	—	Poor.
CXXX.	54	M.C.	Average to poor.
CXXXI.	54	M.C. heavy.	Poor.
CXXXII.	55	—	Poor.
CXXXIII.	56	Haphazard, M.C.	Average.
CXXXIV.	56	Rather heavy but good type.	Average slow but solid.
CXXXV.	56	Feeble mentally and physically.	Average to poor.
CXXXVI.	63	Poor type.	Average to poor.
CXXXVII.	63	—	Average.
CXXXVIII.	66	Slow but sturdy, horse-riding.	Average to poor, very slow.
CXXXIX.	68	Charterhouse School, but slow, heavy.	Poor—very slow.

W. = mistakes in keys.
M. = mentality. A. good, B. average, C. poor.
Where not stated mentality is average.

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FIRST REPORT OF THE COMMITTEE UPON INJURIES OF THE NERVOUS SYSTEM

The Diagnosis and Treatment of Peripheral Nerve Injuries

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THE DIAGNOSIS AND TREATMENT OF PERIPHERAL NERVE INJURIES

CONTENTS

PAGE

INTRODUCTION 5

PART I. NERVE INJURIES IN GENERAL 6

1. Examination and Diagnosis 6
   A. *Motor Functions* 6
      Muscular action 6
      Electrical response 7
   B. *Sensory Functions* 9
      Methods of testing sensibility 9
      Deep sensibility 9
      Sensibility to pin-prick 10
      Sensibility to light tactile stimuli 10
      Heat and cold 11
   C. *Vasomotor, Secretory, and Trophic Functions* 11
   D. *Complications* 13
      Trick movements 13
      Fibrotic changes in joints and muscles 14
      Psychical complications 14
      Reflex paralysis 15
2. Non-Operative Treatment 15
   A. *Postural Treatment* 16
   B. *Nutritional Treatment* 16
      Heat 16
      Massage 16
      Electrical stimulation 17
      Exercises 17
      Re-education 17
3. Operative Treatment 17
   A. *Indications for Operation* 17
   B. *Contra-indications* 18
   C. *Technique of Operation* 19
   D. *Technique of Exposure* 19
   E. *Examination of Exposed Nerve* 19
   F. *Technique of Resection* 20
   G. *Technique of Suture* 21
   H. *Bridging of Gaps* 21
4. Signs of Returning Function 21
5. Pain 24

PART II. SPECIAL NERVES 25

1. Musculo-Spiral Nerve 26
   A. *Diagnosis* 26
   B. *Level of Lesion* 27
   C. *Treatment* 27
      Observation stage 27
      Indications for operation 29
      Operation 30
      Post-operative treatment 32
   D. *Signs of Returning Function* 32

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PAGE

2 Median Nerve 32
A. *Diagnosis* 32
Motion 32
Sensation 33
B. *Level of Lesion* 35
C. *Vasomotor and Trophic Changes* 35
D. *Differentiation between Complete and Incomplete Lesions* . . 35
E. *Treatment* 35
Observation stage 35
Indications for operation 35
Operation 36
F. *Signs of Returning Function* 36
3. Ulnar Nerve 37
A. *Diagnosis* 37
Motion 37
Sensation 38
B. *Level of Lesion* 39
C. *Vasomotor and Trophic Changes* 39
D. *Differentiation between Complete and Incomplete Lesions* . . . 39
E. *Treatment* 39
Observation stage 39
Operation 39
F. *Signs of Returning Function* 40
4. Division of both Median and Ulnar Nerves 40
5. Musculo-Cutaneous Nerve 40
A. *Diagnosis* 40
Motion 40
Sensation 40
B. *Level of Lesion* 41
C. *Signs of Returning Function* 41
6. Circumflex Nerve 41
A. *Diagnosis* 41
B. *Treatment* 41
7. Brachial Plexus 41
Treatment 42
8. Sciatic Nerve 42
A. *Diagnosis* 42
Motion 42
Sensation 43
B. *Level of Lesion* 45
C. *Vasomotor and Trophic Changes* 45
D. *The Relative Vulnerability of Internal and External Popliteal Nerves* . 45
E. *Treatment* 45
Observation stage 45
Operation 46
Post-operative Treatment 46
F. *Signs of Returning Function* 47
9. Internal Saphenous Nerve 49
10. Anterior Crural Nerve 49

PAGE

PART III. HISTOLOGICAL EXAMINATION OF INJURED PERIPHERAL NERVES . 49

1. Injuries of the Nerve Fibres with Intact Nerve Sheath . . . 49

2. Injuries of the Nerve Fibres and of the Sheath 50

General Results of Examination of Completely and Incompletely Divided Nerves 51

PART IV. PAIN WITH INJURIES OF PERIPHERAL NERVES 51

1. Causalgia 52

A. *Symptomatology* 52

B. *Nerves with Injury of which Causalgia is most frequently associated* . 52

C. *Macroscopical Findings* 53

D. *Diagnosis* 53

E. *Treatment* 53

2. The Nervous Sequelae of Amputation 55

A. *Painful Phantom Limbs* 55

Symptomatology 55

Treatment 55

B. *Tender Stumps* 56

Diagnosis 56

Treatment 56

REFERENCES 57

INTRODUCTION

MOST cases of injury to the peripheral nervous system make a good recovery provided that treatment is carried out systematically and over a long period of time. An exact knowledge of what nerve or nerves are injured as well as of the site of the lesion should be arrived at before treatment is begun, and at the same time an accurate record of the motor, sensory, and nutritional changes present should be made. This record has to be supplemented by the results of further periodic investigations in order to obtain a clear idea of the pathological condition of the injured nerve and to regulate treatment accordingly. No rigid plan of campaign should be laid down when the patient first comes under observation; it must be adapted from time to time to the requirements of the individual and to the changes which may be found on examination at different stages.

In the following pages an attempt has been made to enunciate the general principles of treatment, and to indicate the variations demanded by the special physiology and anatomy of each of the more important nerves. Stress has been laid on the need for thorough collaboration between the surgeon, masseur, and patient, and on the fact that no operation, however skilfully performed, can ensure success unless the treatment which precedes and follows it is planned with care and carried out with intelligence.

The Committee desire to express their indebtedness to the Surgical Staffs of the Military Orthopaedic Hospital, Hammersmith, and Tooting Military Hospital, to Mr. Jocelyn Swan, F.R.C.S., Mr. Geoffrey Jefferson, F.R.C.S., and Mr. Welby Fisher, F.R.C.S., for their generous assistance.

PART I. NERVE INJURIES IN GENERAL

I. Examination and Diagnosis.

A. *Motor Functions.*

Muscular action.—When investigating motor functions the examiner first attempts to determine whether a given muscle can be put into action. In a straightforward case, the patient is told to make the appropriate movement and the muscle is seen and felt to contract; here the muscle is said to be acting as a *prime mover.* It may, however, be able to act as a *synergic* or *fixation muscle* when it cannot do so as a prime mover. Thus, after injury to the musculo-spiral nerve, the patient may be unable voluntarily to extend the wrist, although on clenching his fist the wrist is extended. Sometimes the patient may be able to carry out the movement asked for without employing the muscles supplied by the injured nerve and commonly regarded as the sole source of the movement in question. This is known as a *trick movement.*

A common trick movement is that produced by a patient who has learned to make his paralysed muscles and tendons mechanically bring about a change of position through the active movement of a neighbouring joint. For example, a patient who has a complete palsy of the musculo-spiral nerve may produce some extension of the wrist by flexing his fingers at the metacarpo-phalangeal joints. The action of the lumbricals in this way shortens the long extensor tendons, with the result that the wrist is extended.

The reverse of this action may sometimes be noticed in patients with no voluntary power in the flexors of the wrist or fingers, as in a complete median and ulnar lesion. When the wrist is actively extended by the carpal extensors the fingers become passively flexed, so that the patient may even grasp an object between them and his palm, or between his first finger and thumb. The flexor tendons act as ligaments and passively pull down the fingers.

A patient may train himself or be trained to use a muscle to perform an unusual action, when the muscles which are usually employed are paralysed. An excellent example of this can be seen when the wrist joint is flexed by abductor longus pollicis (extensor ossis metacarpi pollicis). This trick is common and has led many observers astray, the movement being regarded as evidence of return of voluntary power in the flexors of the wrist. The action may be quite strong and the wrist may be flexed 30–40 degrees against gravity.

With a complete lesion of the musculo-cutaneous and musculo-spiral nerves, all the usual flexors of the elbow joint are paralysed. But in some patients flexion is still possible, pronator radii teres and the flexors of the wrist and fingers arising from the internal condyle of the humerus producing this action. The elbow may be flexed to 90 degrees against gravity. Such flexion is always accompanied by pronation.

The examination of the small muscles of the hand for voluntary power is often a matter of extreme difficulty, and indeed in many cases no trustworthy information can be gained, the diagnosis resting on other factors such as palpation of the muscles and their electrical changes.

Opposition of the thumb, viz. the action usually performed by opponens pollicis (median nerve), can be simulated by the combined action of the adductors (ulnar) and abductor longus pollicis (musculo-spiral). The thumb can be swept across the palm and at the same time rotated by the combined action of these muscles.

Atrophy of the thenar eminence and the ease with which the external border of the first metacarpal bone can be felt in cases of median nerve injury are more reliable guides than the performance of certain thumb movements.

In examining the action of the interossei it must be remembered that extensor communis digitorum and the special extensors of the index and little fingers are powerful abductors. While abduction of the fingers is being tested the hand and fingers should lie on a smooth flat surface so that the action of the extensors can be excluded.

Electrical response.—This does not suffice in itself for estimating the condition of the nerve under examination, but should form part of the routine in all cases of suspected nerve injury. It is of the greatest assistance in testing certain muscles or muscle groups, such, for example, as the small muscles of the hand and foot and the muscles of the forearm.

The presence of faradic excitability will often immediately clear up any doubts as to the functional or organic basis of a peripheral loss of power.

The limb or part to be examined must be warmed as an immediate preliminary to the electrical tests, and any oedema which is present should be reduced as far as possible by massage. The electrical examination will be quite untrustworthy if carried out on a cold and oedematous limb. Muscle responds more readily to stimulation when warm, and considerable oedema will reduce the conductivity of the limb. A course of massage for a week or ten days should be ordered in doubtful cases, and the electrical tests repeated. Preliminary soaking in a basin of hot water for five minutes should form part of the routine in making the tests.

What is required to be known is the behaviour of the nerve to faradic and galvanic stimulation, and the effect of direct stimulation of the muscle.

The testing outfit consists of a faradic coil, a galvanic battery and resistance board with an interrupting device; a metronome interrupter is the most convenient form to use, but a make and break key will answer all requirements. Two wires lead from the testing table, and a simple arrangement of the switches allows of either the faradic or galvanic circuits being used. In testing, two small electrodes should be employed, either both on the muscle, or one on the muscle and one on the nerve. This method localizes the stimulus and tends to prevent spread to neighbouring muscles.

The nerve and the muscle are first tested for faradic excitability, and, if this is present, no further electrical examination is necessary.

If no contraction can be obtained with the faradic, the switch is turned and the galvanic current used. The two electrodes should be placed longitudinally on the muscle which is being tested, or one electrode be placed on the muscle and the other on its tendon. The intensity of the current is gradually increased ; but, if this must be done to such an extent that neighbouring muscles contract, the current need not be further increased. To the galvanic current a normal muscle responds with a brisk twitch, and when a tracing is taken with a myograph the sudden contraction and relaxation are well shown.

When conductivity in the nerve is completely destroyed, the phenomenon known as the reaction of degeneration is manifest. The reaction of degeneration is to be defined as an absence of

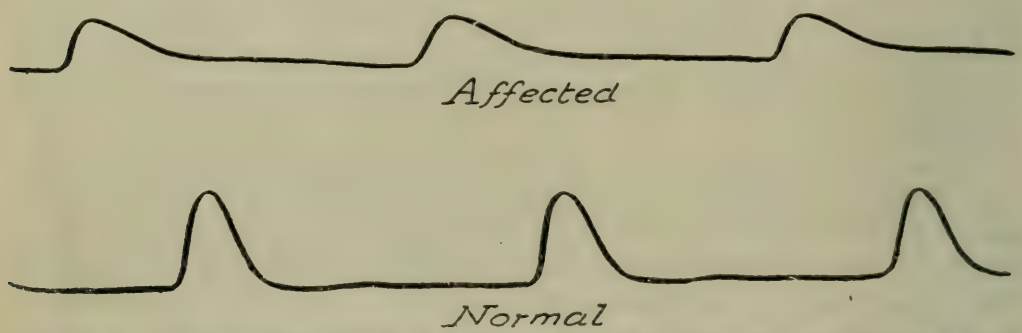


Fig. 1. The descending limb of the curve from the affected muscle shows a more gradual descent than that from a normal muscle.

faradic response when either the nerve or the muscle is stimulated, with a change in the form of galvanic response in the muscle from a brisk twitch to a slow contraction. Myographic tracings (Fig. 1) show clearly that this slowing of the galvanic response is chiefly due to a prolonged relaxation period.

Much stress is sometimes laid on polar changes, but these are inconstant and have little or no clinical value. The presence or absence of faradic response is the really important factor and in the great majority of cases forms a trustworthy index of the condition of the nerve.

In rare instances a faradic response is obtained with a current of only moderate strength, even when the nerve is known to be divided. The irritability of the muscle may be such that an electrical stimulus of short duration, as obtained from a faradic coil, will cause it to contract. This has been seen several times in the case of tibialis anticus. Therefore in the case of this muscle electrical findings which are at variance with evidence gained from other parts of the examination should be disregarded.

An improvement in the galvanic response may be brought about by nutritional treatment in the absence of any nerve recovery. The state of the muscle improves with massage, heat, and galvanic stimulation, but this must not be regarded as evidence of definite recovery in the nerve.

B. *Sensory Functions.*

Methods of testing sensibility.—When testing the condition of sensibility for the purpose of clinical diagnosis, the simplest methods should be employed. But in order that the results obtained by such means may give accurate information, the significance of these tests must be understood. The use of the camel's hair brush, cotton-wool, and the pin-point is liable to lead to fallacious conclusions unless the observer bears in mind that they are essentially tests capable of yielding only the broad qualitative results required for practical diagnosis.

Misleading and confused results may also be obtained unless some thought is given to the physical and mental environment of the patient. The bodily requirements are that the patient should be comfortable and his limb warm. Psychologically the commonest sources of error are fatigue and misunderstanding, or even unconscious antagonism between patient and observer. The observer must be systematic and prompt, carrying through the examination without unnecessary and tedious repetition, and should be sparing and gentle in the use of painful stimuli. He should point out to the patient the necessity of screening the part to be tested, and make clear that there is no desire to ' catch ' or puzzle him. The broad principles of the examination should be explained without suggesting the form of his answers. All such questions as ' Did you feel that ? ' ' Did I hurt you ? ' ' Is that hot or cold ? ' must be avoided.

First, the patient is asked to give an account of any spontaneous sensations which he experiences as the result of the nerve injury. If he suffers from pain, its distribution should be recorded on a chart, and it is important to note how these sensations behave to changes in external conditions.

Deep sensibility.—Deep sensibility may be defined as the power of appreciating and localizing the pressure, whether painful or not, of a blunt instrument. It may be preserved over parts where the patient is unable to appreciate the sharpness of a pin or the touch of cotton-wool. For example, after complete division of the median nerve at the wrist, the terminal phalanges of the index and middle fingers may remain sensitive to firm contacts; and, if so, discomfort can be evoked by increasing the pressure. The fibres of the median nerve, on which this deep sensibility depends, are given off in the forearm and pass into the fingers by way of the long tendons. Presence or absence of sensations of pressure may be therefore of considerable diagnostic importance, especially if the wound is situated at the wrist.[1]

Another function which depends on the presence of deep sensibility is the power of recognizing posture and passive movements of the various joints. It is well, especially with lesions affecting the upper extremity, always to carry out these tests ; but they are usually of corroborative rather than of primary diagnostic importance. Thus, should the patient lose the power of recognizing movement at the

[1] For if the tips of the middle and index fingers remain sensitive to pressure, these fibres have escaped and the tendons are probably intact, while if deep sensibility is absent, they are likely to have been divided.

terminal joints of the index and middle fingers, after a complete lesion of the median nerve, he will also be unable to appreciate pressure, painful or not, over the same parts.

Sensibility to pin-prick.—Next an attempt is made to determine what parts are completely insensitive to the prick of a pin. The boundaries of this area are not as a rule well defined, but merge gradually into portions where sensibility is present, though grossly defective. This adds greatly to the difficulty of recording the results on a chart.

The limits of this analgesia differ from time to time, and are particularly liable to vary with vascular changes of the skin. Sometimes, when testing with a pin, the patient gives a positive answer, although in reality he has no sensation of prick, but recognizes the pressure exerted by the point. The examiner must be certain that the patient is responding to the painful or uncomfortable elements of the test.

Then the affected limb is examined for areas of tenderness. In some cases of nerve injury these are not present, and the loss of sensation passes rapidly into parts of normal sensibility; but after even complete division of a peripheral nerve, such as, for example, the median, it is rare for the whole of the area assigned to it anatomically to be insensitive to the prick of a pin. In such a case, when a pin is drawn gently across the palm from the ulnar region, the patient usually flinches as soon as the radial side of this anatomical border is passed. He complains that 'it hurts more', and that the 'feeling runs into my fingers'. Otherwise he may say, 'It is numb, but it hurts.'

This condition is usually spoken of as 'hyperaesthesia'; but careful measurements have shown that, at any rate with complete division of the nerve, the area of tenderness is one of diminished sensibility associated with over-reaction to uncomfortable stimuli. In other words, the stimulus necessary to cause a sensation of pain is greater than normal, although, once evoked, the sensation is more disagreeable. Many patients can make out this border for themselves by passing a finger-tip of the normal hand across the palm and noting the points at which the sensation changes. The over-reaction to all forms of disagreeable stimuli fades irregularly into parts completely insensitive to prick. This is the condition commonly found in the hand some ten days or more after division of either the median or the ulnar nerve. Moreover, it is usually a prominent feature during the return of sensibility, especially to the palm of the hand or sole of the foot.

Sensibility to light tactile stimuli.—It is important to remember that unless the camel's-hair brush is carefully used it may excite deep sensibility, and may be appreciated over parts where the surface is entirely insensitive to the lighter degrees of touch. A similar fallacy also accompanies the use of cotton-wool, if it is balled into a swab and used by 'dabbing' the part. Again, cotton wool is not infrequently an insufficient stimulus to the horny hand of a manual worker.

The effect produced by cotton-wool and the camel's-hair brush is greatly increased by the leverage of the hairs. The presence of

sensibility to these stimuli over hair-clad parts is therefore not equivalent to its presence over portions devoid of hair, such as the palm of the hand and sole of the foot. After complete division of the ulnar nerve, the patient can sometimes appreciate contacts with cotton-wool on the back of his hand for a considerable distance within the borders usually assigned to this nerve; but on the palm the loss to cotton-wool almost exactly corresponds to the anatomical boundaries. This difference depends on the existence of the hairs on the dorsal surface of the hand; it disappears when they are carefully removed by shaving, and the extent of the loss of sensation to these lighter tactile stimuli then considerably exceeds, in most cases, the complete loss of sensibility to pin-prick. A similar difficulty occurs in defining the upper limits of the external popliteal supply by means of cotton-wool, unless this part of the leg is shaved.

The increased stimulating effect of cotton-wool, due to the presence of hairs, is still more evident during recovery; hair-clad parts regain their sensibility to cotton-wool and the camel's hair brush almost at the same time as they become sensitive to the prick of a pin.

Heat and cold.—Tests with heat and cold are difficult to carry out with lesions of peripheral nerves, and they contribute little of practical value.

Extreme degrees of heat and cold, such as ice, and water at 50° C., excite discomfort and are liable to evoke the same over-reaction as painful stimuli. This is especially evident in parts where sensibility is recovering after complete division of one of the larger peripheral nerves.

C. *Vasomotor, Secretory, and Trophic Functions.*

Investigations into vasomotor, secretory, and nutritional disturbances form a part of the routine examination in injuries of peripheral nerves. The underlying factor in changes of this order is probably vaso-dilatation, brought about by destruction of the constrictor fibres in transection of a motor or a mixed nerve. Though vasomotor and nutritional alterations are usually present in some degree when conduction is abolished in any of the larger nerve trunks, they are most pronounced with injuries of the median, ulnar, and sciatic nerves.

When a nerve is completely divided, that part of the skin which is analgesic becomes inelastic, puckered, and does not sweat. It feels colder than the surrounding healthy skin and desquamation is retarded, so that the surface becomes scaly and even loaded with heaped-up epithelium. In the colour of the skin there is always a bluish tint which becomes deeper in cold weather or when the part is allowed to hang down. Dependence of the limb leads to swelling. In the analgesic area the tissues are liable to injury, and, unless the part is protected, ulcers which are slow in healing readily develop. Most of the so-called trophic sores are obviously the outcome of injury to the skin and defective nutrition, but occasionally small blisters appear and break down, leaving an ulcerated surface. As soon as sensibility to pain returns accidental wounds heal

readily and the skin regains its elasticity, and cyanosis begins to disappear.

Diminished growth in the nails after division of the peripheral nerves to the hand largely depends, as Head and Sherren (5) have shown, on immobilization of the hand and fingers, either from muscular paralysis or from splinting. Adhesions between tendons and their synovial sheaths, and fibrous alterations in the muscles and joints, are due to the same cause, for they can be prevented by the daily practice of massage and passive movements. Immobility, particularly of the peripheral segment of a limb, leads also to cyanosis and oedema. Vasomotor disturbances in hysterical paralysis, and those associated with splinting, result from loss of movement.

Pressure from badly applied splints may be another factor in bringing about grave changes in the tissues. When mechanical means have to be employed to prevent stretching or contraction of paralysed muscles of the hand or fingers, it should be remembered that the vessels passing into the hand are but slightly protected against pressure by the soft tissues of the wrist. Care must therefore be taken to employ splints which fit accurately and exert only light pressure on the tissues ; they must be removed at frequent intervals to allow of movement of the hand and fingers. Whenever possible the fingers and thumb should be left out of the splint and the patient encouraged to use them.

It should always be kept in mind that gross changes in nutrition, resulting in wasting and ulceration of the soft tissues, atrophy of bones, and fibrous ankylosis of joints, may be directly vascular in origin. Large blood-vessels lying alongside the nerve may have been partly or completely occluded as the result of injury and with the consequence that the blood-flow to and from the periphery of the limb is impeded.

With complete nerve division vaso-dilatation is a paralytic phenomenon. But when the interruption in conduction is incomplete and the injury is associated with severe and continuous pain, as in causalgia, dilatation of the vessels is probably the result of stimulation of the vaso-dilators. The nerves with injury of which causalgia is usually associated are the median, sciatic, and ulnar. Sensibility is not abolished ; there is little or no paralysis and the hand and foot may become gravely deformed.

In extreme cases of causalgia the skin is thin, smooth, and glossy. The cutaneous folds disappear, and the surface may sweat profusely. In those cases in which the skin remains dry, the constrictors are probably destroyed. The skin may look like wash leather owing to the fact that the patient tends to keep the hand continually moist with water in order to obtain relief from the pain. Sometimes the skin is bright red in colour or mottled red and white. It may be tense and firm as if tightly stretched over the wasted tissues. Small vesicles containing clear or blood-stained fluid may be dotted over the surface of the hand and fingers, and chilblains are common. Characteristic changes occur in the nails. Curved longitudinally and transversely, they grow more rapidly than the nails of the normal fingers ; they are often thin, striated, and exquisitely tender, and the sensitive nail-beds protrude because of wasting of the finger-

pads. The bones become decalcified and brittle and the fingers tapering. Movement of the hand and fingers can scarcely be tolerated, and, in consequence, it is extremely difficult to combat the development of fibrous changes in the joints and muscles, which often leave serious deformities.

In the more chronic cases the appearance of the hand or foot may entirely change. The ruddy colour of the skin may be replaced by pallor, and sweating is no longer observed. The affected muscles become hard and waste rapidly, in spite of daily massage, and gangrenous patches may appear within the distribution of the nerve. These changes suggest arterial obstruction as the underlying cause. The histological examination of the peripheral arteries of the foot by Stopford (7) in a case in which the sciatic nerve was injured without concomitant damage to any of the large arteries of the limb, showed extensive thickening of the intima with great narrowing of the lumen of the vessels.

D. *Complications.*

The need for careful investigation of cases of peripheral nerve injuries is well illustrated by a brief reference to certain important complications which are apt to lead the observer astray in arriving at a diagnosis. It may show that a voluntary movement, recently acquired by a patient and regarded as evidence of recovering function in an injured nerve, cannot be accepted in that light. On the other hand, it may show that the failure to perform a voluntary movement cannot be ascribed to an injury of a peripheral nerve, but requires some other explanation.

In the first instance we have to deal with trick movements, and in the second instance with such widely different complications as arthritic adhesions and hysteria.

Trick movements.—It must be remembered that the brain is concerned with voluntary movements and not with muscles, with bringing about an alteration in the position of a limb or a segment of a limb in relation to other parts of the body. In attaining this object there is no conscious or deliberate attempt to make a muscle or group of muscles contract. Unconsciously certain muscles are put into action, and the required alteration in position becomes an accomplished fact. If one or more of the muscles usually co-operating in this movement are put out of action by damage to a peripheral nerve, the patient is at first unable to make the desired alteration in position. He will, however, naturally make experiments and, after a varying period of time, may improvise a movement producing an alteration of position in the limb which satisfies, or nearly satisfies, his desires. It is this improvised movement which has to be distinguished from recovery of function in an injured nerve.

Trick movements have already been considered on p. 6, but another specific instance may here be taken as an example. In an Erb's palsy, due to injury of the fifth and sixth cervical nerves, there is often complete paralysis of biceps, brachialis anticus, and supinator longus muscles, and the patient is unable to bend his elbow. In the course of time he learns that after pronating his forearm he is able to bend his elbow, and the observer will find that

he is making use of his wrist extensors to attain this end. The recognition that this is a trick movement is important for two reasons. In the first place it avoids error in diagnosis and perhaps in treatment. In the second place it should prevent the patient from relying entirely on the abnormal movement, and from neglecting those constant voluntary efforts which are so necessary in securing the recovery of muscle function after nerve injuries.

Fibrotic changes in joints and muscles.—The failure to perform a movement may be the result of arthritic adhesions or of interstitial myositis. The former condition may be due to errors in treatment and especially to prolonged immobilization with splints. It is also of frequent occurrence in association with partial injuries of nerves, the best example being that provided by the median. In cases of median causalgia the metacarpo-phalangeal joints very rapidly become fixed by adhesions, with the result that no movements are possible, even when the pain has subsided and the long flexors of the fingers have recovered their functional activity.

Fibrotic changes in muscles may be due to prolonged sepsis or to interference with their vascular supply. The latter (ischaemic paralysis) is sometimes brought about by faulty splinting, and at other times by the destruction of the main arterial supply of the limb. Careful investigation of the muscles, including their response to both volitional and electrical stimuli, is necessary in all cases in order to determine the cause of their failure to produce a movement, and no exploratory operation on the nerve should be performed until this has been done.

Psychical complications.—It is not uncommon to find defects of movement without any serious affection of the neuro-muscular tissues when the limb has been immobilized for some time, or when there has been a severe nerve injury which has recovered with or without operation. This failure on the part of the patient to make use of the power he possesses must not be mistaken for hysteria. It is a functional but not an hysterical disorder, and is one which can be rapidly cured by explanation and re-education.

Hysterical palsies are more easy to differentiate from peripheral nerve palsies and often more difficult to cure. They originate consciously or unconsciously in the mind, and vanish only when the idea suggesting them has been dispelled. It is characteristic of hysterical palsies that a muscle which fails to contract in the attempt to make an active movement may yet display its integrity in passive resistance, or when exercising its synergic function. The patient with an hysterical dropped wrist, for instance, will throw his long extensors of that joint into action when exerting his grasp. The hysterical disability may be more complex. For instance, a professional boxer received a bullet wound in his arm, which caused no serious damage to nerves, muscles, or bones. He was able to use the hand for all purposes with one exception, that of making a fist.

In many cases the originating idea behind the disability is not far to seek ; in others it requires long and careful search before pyscho-therapeutic measures are successful.

It must not be forgotten that organic and hysterical disabilities are sometimes associated in the same limb, and it may be a matter

of some difficulty to determine how far each of these causes is responsible for the result.

Reflex paralysis.—This title, which rather begs the question of pathogenesis, has been applied by Babinski, Froment (1), and others to a group of cases closely allied to the hysterical complications of gunshot wounds. The symptomatology, which has been fully described by the above authors, includes a large number of phenomena, not all of which are present in every case. The lesion with which this clinical picture is associated is often slight and out of proportion to the resulting disturbances of function. The latter tend to spread in a manner which cannot be reconciled with our knowledge of anatomy and physiology, when the site and nature of the original injury are taken into account.

The affected limb, which will often have been immobilized for a varying length of time by means of bandages or splints, tends to assume a fixed and unnatural posture, and if untreated in the early stages develops contractures, adhesions, and disorders of nutrition, associated with disturbances of motion, sensation, and vasomotor control.

There may be atrophy of muscles which are often hypotonic yet hyperexcitable to mechanical stimuli and do not show the reaction of degeneration. Cutaneous reflexes are generally diminished or abolished, while tendon jerks display increased activity. The periphery of the limb is usually cold, cyanotic, and sometimes sweating. The bones, nails, skin, and hairs share in the general disturbance of nutrition. The growth of hair may be apparently increased owing to the immobilization of the limb and absence of friction. Sensory disorders may be present in the form of pain or anaesthesia, but they cannot be correlated with any anatomically grouped areas of nervous distribution.

Such symptoms have often to be dealt with after the disorders have been long established, and are apt to prove resistant to treatment. This is perhaps not a matter for surprise, seeing that the conditions represent the results of both psychopathic and physiopathic influences. When analysed, they generally present some features which are hysterical, and others which are clearly the results of immobilization. It is important to remember that the latter may not only be brought about by splints and other mechanical means, but may also have an hysterical origin or result from inhibition when movements of the affected parts have been accompanied by pain. Though complex in their origin these cases do not need the assumption of an elaborate hypothesis of reflex action in order to explain their pathogenesis.

2. Non-Operative Treatment.

The treatment of cases of peripheral nerve injury during a period of observation and after operation is on similar lines.

It may be considered under two headings :

A. Postural treatment.

B. Nutritional treatment.

A. *Postural Treatment.*

The principle of treatment by posture is to relax the paralysed muscles and so avoid their being stretched either by the force of gravity or the pull of the non-paralysed opponents. The position of relaxation which is necessary is dealt with under the individual nerves. It suffices here to mention that the joints will not get stiff in the positions advised and with proper precautions. The shoulder which is held abducted for several months will adduct readily shortly after the splint is removed. The foot which has been held in dorsiflexion for a long period can readily be extended. With regard to the hand, fixed extension of the wrist joint will not be followed by a stiff wrist, but the fingers must be allowed free movement, both at the metacarpo-phalangeal and inter-phalangeal joints, and the thumb must be kept abducted and extended. The small joints of the hand will get stiff if kept at rest for a few weeks, and this stiffness is more easily prevented than cured. In most cases there is no need to remove the limb from the splint, and the treatment can and should be carried out with the muscles relaxed.

In the case of the abducted shoulder, all passive movements that are possible without diminishing the degree of abduction should be performed.

It is scarcely necessary to say that splints must not be employed indefinitely, and when no further recovery can be expected, the use of them should be abandoned. This, of course, does not apply to splints used not for treatment but to assist function.

B. *Nutritional Treatment.*

The treatment which is necessary in order to maintain and improve the nutrition in the paralysed part comprises :

Heat.
Massage.
Electrical stimulation.
Exercises.
Re-education.

It will be necessary to modify the treatment in cases complicated by trophic or pressure sores, which cannot, for instance, be soaked in hot water. Again, when severe pain is a prominent symptom, the treatment must be modified. If heat causes pain it should not be employed.

Heat. Soaking the limb in a tub of hot water is at least as good as any other method of applying heat and especially when employed as a preliminary to electrical stimulation. Among the other methods used may be mentioned the whirlpool bath, in which the water is kept in rapid motion by a propeller; radiant heat baths, diathermy or electro-thermo-penetration, and the paraffin bath, in which the limb is immersed in molten paraffin at a temperature of 130° F., can all be used.

The limb must at all times be kept warm, and extra stockings, or long woollen gloves, as the case may be, should always be used in cold weather.

Massage.—Massage should be carried out once daily for 15 to 20

minutes and must never be sufficiently vigorous to cause pain. It is the best physical method of reducing œdema.

Electrical stimulation.—The limb must be heated, preferably in a tub of hot water, as an invariable preliminary to electrical treatment.

Electricity is of value only as a stimulus to provoke contractions in the paralysed muscles. The weakest effective current, galvanic or faradic, should be employed. The two terminals are applied over each individual paralysed muscle in turn and a minimal current will obviate the spread to neighbouring healthy muscles. The practice of passing a current through the water in which the limb is immersed is to be condemned. Such a method will more readily excite contractions in muscles other than those requiring treatment, and this is directly harmful.

Exercises.—When recovery of voluntary power is commencing, passive movements of joints should be replaced by active exercises which are at first assisted by the masseur and later on carried out against resistance. 'Assisted exercises' are those in which the masseur actively helps the patient to make the desired movement.

Recovering muscles must not be fatigued. It is always better to do too little rather than too much, and, if the newly-gained power of movement is lost, it probably means that too much work is being done by the weak muscle.

Re-education.—This is perhaps the most important part of the treatment. The masseur's duties do not end with merely rubbing the limb and electrically stimulating the muscles. It is an essential part of the work to interest the patient in his recovery, and as regeneration advances to teach him to reproduce voluntarily those movements which have been provoked by electrical stimulation.

Generally speaking, large muscles, such as the flexors of the elbow or knee, will recover their function, without any special training, as the nerve regenerates. Specialized re-education becomes particularly necessary when we are dealing with the intrinsic muscles of the hand.

During the early stages and before any voluntary power can be expected, the patient should be made acquainted with the movements of joints and their range. He should not at this stage make active efforts to produce the movements normally carried out by the affected muscles, for if he does he is liable to acquire trick movements which stretch the paralysed muscles and will learn habits which ultimately impede function.

It is common to find patients who are unable to initiate movements with the affected muscles, although there is evidence that their nerves have recovered. If skilled re-education has formed a part of the treatment, this condition of functional paralysis will be avoided.

3. Operative Treatment.

A. *Indications for Operation.*

With few exceptions operations upon injured nerves must necessarily be of an exploratory nature. Clinical observation can determine the site of the lesion and the presence or absence of the conductivity in the nerve. In many cases the condition revealed when the nerve

is exposed can be foretold with some accuracy; but it is only by inspecting, handling, and directly stimulating the nerve that its state can be ascertained with precision. This particularly applies to cases where from clinical evidence the lesion appears to be incomplete. The primary indication, then, for operation in doubtful cases is to determine the nature of the structural changes after every other means of arriving at a precise diagnosis has been employed. It must not be thought that surgical exploration should ever do more than supplement careful clinical observation.

Operation is generally indicated in the following circumstances:

(*a*) Total loss of conductivity, sensory and motor, in the territory exclusively supplied by a nerve, persisting after an interval of two months during which proper treatment has been carried out. This interval is an arbitrary one; it allows time for the first appearance of signs of recovery, provided the lesion does not necessitate the lengthy process of regeneration.

(*b*) Palpable neuromata at the site of injury of a nerve the function of which is seriously disturbed.

(*c*) When recovery has begun but has not progressed according to the usual rate or has actually ceased. Still more when function has relapsed.

(*d*) Persistent, severe, intractable pain (see Causalgia, p. 52).

With the reservation that a longer interval of time must be allowed, all these indications for operation apply with equal force to a nerve which has already been operated upon, more particularly if there should be any doubt as to the exact nature of the operation which has been performed. Experience has provided ample justification for this recommendation. In the first place some useless procedure may have been adopted, such as lateral implantation, lengthening by means of flaps cut from the nerve, interposition of strands of catgut or pieces of vein. An attempt to approximate divided ends may have been abandoned; technical errors may have been committed such as suture of cut nerve to a divided tendon or a ligature placed round nerve in mistake for artery. Further, a nerve which should have been sutured may have been merely freed, or a nerve may have been wrapped round with some material which causes such tissue reactions as retard or prevent recovery. In the second place a nerve which has been correctly dealt with by freeing or suture may fail to recover, and require some further direct treatment.

B. *Contra-indications.*

It is, perhaps, easier to make definite general rules as to contra-indications than as to indications for operation.

(*a*) The presence of an unhealed wound should be regarded as an absolute bar to successful operation upon an injured nerve. When the wound has been healed for a few weeks only, especially if bone was damaged at the time of injury, the operation should be delayed on account of the danger of recrudescent sepsis. Fortunately such delay has little appreciable effect upon the ultimate result.

(*b*) Progressive recovery contra-indicates operative interference.

It must be remembered that the early signs of recovery may be associated with increased discomfort.

(*c*) When the injury is so placed as to render an operation unusually difficult, or the likelihood of successful suture very doubtful. For example when the great sciatic nerve is damaged within the pelvis, it is advisable to defer operative interference for a relatively longer period than would be justifiable in the case of a more easily accessible nerve.

C. *Technique of Operation.*

It is essential that the operator should be familiar with the exact normal anatomical relations of the nerve which he contemplates exposing. The cutaneous incision must be so planned as either to deal radically with or to avoid the scar of the original wound, whilst at the same time it should provide free access to the damaged nerve for a considerable distance both above and below the site of injury. Asepsis must be rigidly maintained throughout, and to this end the probable necessity for changing the position of a limb during the operation must be foreseen. The operation must be conducted as far as possible in a bloodless field. Bleeding constitutes one of the chief difficulties, and to avoid this some surgeons adopt the Esmarch bandage or tourniquet. There is a serious objection to this method, however, in the oozing which is likely to take place later into the tissues around the nerve. A post-operative haematoma not only endangers the asepsis of the wound, but is likely to result in the formation of a harmful amount of cicatricial tissue.

D. *Technique of Exposure.*

No attempt to examine the site of injury should be made until the nerve has been exposed both above and below that point. Before the damaged part is disturbed, the trunk above should be freed, insulated, for instance by passing a broad strip of dry india-rubber beneath it, and stimulated with an extremely weak faradic current, and the effects noted and recorded.

E. *Examination of the Exposed Nerve.*

In many cases the discontinuity of the nerve is so obvious that it is clear at a glance that resection of the end bulbs and suture must be done; then little good can come of electrical examination. Yet it is interesting to note that definite motor effects, not of a reflex character, can often be obtained in a case of this kind. This may be due to the stimulation of axons which have chanced to remain or have succeeded in bridging the gap. Sometimes a response is obtained from stimulation of the peripheral end of the nerve, a result which may be due to the ingrowth of axons from injured nerve twigs in the neighbourhood. The importance of this observation lies in the fact that it serves as a warning against over-estimating the value of a response to electrical stimulation in a nerve which may appear to the naked eye to be so grossly damaged as to require resection.

In examining the exposed nerve by inspection and palpation,

a note should be made of any abnormality. Constriction by scar tissue of a nerve which otherwise does not appear to be grossly damaged is a condition for which a mere freeing of the nerve is probably sufficient.

A neuroma which involves a part only of the thickness of the nerve, or an intraneural neuroma, may be seen: occasionally a spicule of bone or a fragment of metal may be found embedded in the nerve. Such localized lesions as these cause little difficulty; they call for resection and suture.

It is otherwise with the more diffuse effects of injury, as when the nerve is swollen and thickened for a considerable distance above and below the site of the damage. In such cases the nerve may do well if freed, or placed in a more favourable position. As a general rule, a damaged nerve which exhibits diffuse naked-eye changes, but is found to possess any conductivity when stimulated with a faradic current, should merely be freed, whereas in the presence of a definite localized lesion resection is necessary even if some slight conductivity can be demonstrated electrically.

In regard to the questions discussed above, the functions of individual nerves have to be taken into consideration and these are considered in the next chapter.

F. *Technique of Resection.*

The necessity for gentle manipulation cannot be too strongly insisted upon. Delicate forceps, sharp knives, and the smallest possible needles must be used.

There has been considerable discussion as to whether the whole or a part only of the proximal bulb should be resected. What is probably of more importance is that the sectional area of the two surfaces which are to be sutured together should be as nearly as possible of the same size, and that individual nerve bundles should be distinctly visible. That further resection would increase the difficulty of approximation is no valid reason for suturing together unsatisfactory surfaces. The various special manœuvres for bringing together widely separated ends which may be adopted in the case of individual nerves are mentioned elsewhere. Some methods of general utility are:

(1) Loosening of the nerve in its bed for some distance, both above and below, by passing a blunt dissector along it whilst gentle traction is being exerted. A valuable gain in length can be obtained in this way.

(2) Relaxation of the nerve by flexion or extension of the joints over which it passes.

(3) Displacement of the nerve, so as to make it pursue a more direct course.

(4) A two-stage operation. If it is clearly recognized that resection would leave a gap of unusual magnitude, the two bulbs should not be resected at this operation but tied firmly together, whilst the whole nerve is relaxed as much as possible by placing the limb in a suitable posture. The wound is then closed. A few days later the gradual straightening of the limb is begun, and at the

end of two or three weeks, when the limb has been completely straightened, the nerve should again be exposed. It will then, in all probability, be found that enough elongation has been obtained to allow of end to end approximation after resection of the bulbs.

G. *Technique of Suture.*

Material.—Whatever material is used, it should be employed in as fine a size as is consistent with efficiency. It should also be a material which excites the minimum amount of fibroblastic reaction around it. Fine linen thread and silk make satisfactory suture materials. Catgut, as ordinarily prepared, causes much more cellular reaction than either linen thread or silk, and it is doubtful whether this disadvantage is compensated for by its greater absorbability. The passing of a central retention stitch through the thickness of the nerve is undesirable, and rarely necessary. A proper approximation can be secured by suturing the edges of the sheath only. A continuous suture should never be used. It interferes with the escape of reactionary exudate from the interior of the nerve, and is also likely to produce a stricture. The two surfaces should not be brought tightly together; regeneration takes place more easily when a minute interval separates them. After suture the nerve should, if possible, be left lying in contact with normal cellular tissue or muscle. No advantage has been shown to attend the wrapping of the nerve in artificial membranes, fat, or fascia, and subsequent explorations have not infrequently shown that these materials in some cases do exert a harmful influence. The wound must be rendered as dry as possible before being closed. The importance of thorough haemostasis can scarcely be overrated. Whilst drainage can usually be rendered unnecessary by careful haemostasis, there is no particular reason why it should not be employed in a special case.

H. *Bridging of Gaps.*

When it is impossible to obtain approximation of the ends of a divided nerve, the outlook is extremely bad, for there is at present no known method which can take its place. It is therefore of the utmost importance that every possible manœuvre should be employed and every means exhausted for securing end to end approximation before trying the last resort of grafting. Lateral implantations and flap operations are only mentioned here to be condemned. There is nothing in what is at present known of the processes of regeneration of nerves to justify them, and no unimpeachable evidence that they have even been successful. Bridging of the gap with a piece of nerve, whether living or alcoholized, is justified on histological grounds, by experimental work on animals, and by a few clinical cases of very imperfect recovery. But in the present state of our knowledge nerve grafting ought to be regarded as a last resort.

4. Signs of Returning Function.

Return of function after nerve-suture depends, in the first place, on the re-establishment of structural continuity between the fibres of the proximal end of the nerve and the peripheral structures.

Neuro-fibrils appear to grow at a fairly constant rate, so that the length of time required for regeneration of a given fibre varies directly with the distance it has to travel. The shorter the course the sooner will connexion be accomplished. It follows, therefore, that, in general, recovery can be expected earliest in short nerves, in cases in which the lesion is distal, and in those muscles the motor points of which lie nearest to the site of suture. The table on p. 32 gives some illustrative figures for the musculo-spiral nerve.

But the recovery of function is under the control of factors other than those concerned merely with regeneration of the nerve-fibres. The care with which the paralysed parts have been attended to during the period of waiting, before and after the operation, has a profound influence on the time taken for the return of function after suture as well as on the completeness of the ultimate recovery. Results will inevitably be disastrous, however perfect regeneration of the nerve may be, if the affected muscles are allowed to become wasted and stretched and the skin to remain poorly nourished from the neglect of appropriate nutritional and postural treatment. Another essential factor for the recovery of voluntary power, and one on which too much stress cannot be laid, is the necessity for making the patient realize that a successful result cannot be hoped for if he adopts the attitude of the passive onlooker with no responsibility in the work to be done. From the time that recovery begins to appear he must take an active and leading part in the daily treatment by trying, through voluntary effort, to bring his affected muscles into action. His attempts at first require to be under careful supervision in order that the development of trick movements may be avoided.

The degree of specialization of function in different nerves and in separate muscles supplied by the same nerve has a prominent bearing on recovery. The median nerve, for example, requires a longer time than the musculo-spiral and the intrinsic muscles of the hand than those of the forearm ; and recovery on the whole is less perfect. Illustrations of this rule can also be drawn from the order of return of sensibility to different stimuli.

The interval between the wound and the operation has no influence on the time taken for recovery, for as good functional results have been obtained from suture performed two or three years after the date of the injury as from primary suture.

In 1909 Trotter and Davies (9) recorded the phenomenon of peripheral reference of sensation produced by mechanical and other forms of stimulation of a reunited sensory nerve below the point of suture. It was observed first between the tenth and fourteenth week after the operation. They pointed out that the phenomenon they described differed in two essential respects from the effects of mechanical irritation of a normal nerve. In the first place the response could be obtained with abnormal ease, and in the second place the sensation evoked was in itself abnormal ; it spread widely in the territory of the nerve and has a peculiar tingling quality.

These observations were corroborated by Tinel (8) and others during the recent war, but undue stress has been laid on the importance of

them as indicating functional recovery in the nerve. It is necessary to state what, in our opinion, is their correct signification and value.

(1) When this phenomenon is present we believe that it indicates the existence of active nerve fibres at the point stimulated, but it gives no information as to their number. The ' bulk ' of sensation originated bears no relation to the number of fibres stimulated.

(2) When the point of stimulation is well below the site of injury, it is direct evidence that the nerve is no longer discontinuous.

(3) If it is elicited at the end of two months a further delay of two months is justifiable in the hope that some sign of returning sensation may make its appearance. If at the end of that time this phenomenon stands alone the nerve should be explored, the surgeon of course being prepared to close the wound if the nerve is found in a condition satisfactory for recovery.

(4) Unfortunately it is necessary to lay stress on the fact that the presence of this phenomenon is no guarantee that further recovery will ensue, and still less that the recovery will be complete.

The nerve should be percussed from below upwards, and care should be taken that an impulse is not transmitted through scar tissue to the central end of the nerve. The phenomenon is of value only when it can be elicited by gentle stimulation.

The first evidence that function is returning, after the reunion of the ends of a mixed nerve, is usually volunteered by the patient. He becomes aware of some change taking place in the distribution of the nerve. He says that the part ' feels different ' or ' is more life-like ', that it ' tingles ' and is less ' numb '. Very soon after this record has been made, definite alterations in the extent and nature of sensory loss can be ascertained by examination. The first alteration in sensation to be detected is in the appreciation of pressure. The area of loss of deep sensibility gradually diminishes. Pin-pricks are responded to before light touches and, at a certain stage in recovery, the sensation evoked by a prick is diffuse, badly localized, and is more disagreeable than that caused by pricking the normal skin. About the time that sensibility to pin-prick returns the skin loses its cyanosis and begins to look more healthy. There may be considerable improvement in cutaneous sensibility before any sign of functional recovery in the muscles is apparent.

Sometimes restoration of faradic excitability precedes voluntary muscular contraction, but this order is by no means invariable. To determine whether certain muscles, such as abductor brevis pollicis and flexor carpi ulnaris, take part in a voluntary movement, is notoriously difficult, and their functional activity may be more accurately tested by the faradic current. While investigating the actions of muscles in peripheral nerve injuries, it should be kept in mind that a muscle may be employed in more than one way in the performance of a voluntary movement. It may be one of the prime movers of the segment of the limb to be displaced, or it may act as a synergic or a fixator or an antagonist. As Beevor (2) originally taught, each of these separate actions should be tested, because at an early stage in recovery the contraction of a muscle may be more easily demonstrated when it is employed in one way than when it is employed in another. For instance, recovering muscles can often

be used to fix a part placed passively in position before they have the power to move it. In testing for the action of muscles as prime movers, it is best at first so to arrange the segment of the limb to be moved that the muscles are not opposed by the weight of the segment.

5. Pain.

Pain of any considerable severity is an infrequent consequence of injuries to peripheral nerves. The soldier who has been wounded usually says that ' the limb became numb ' or that ' it felt as if it had been shot away '. After the first shock has passed off, the parts innervated by the injured nerve are found to be insensitive, and the muscles it supplies are paralysed. In the early stages of recovering sensibility parts such as the hand and foot may become ' sore ' and ' tender ', in consequence of their peculiar over-reaction to all unpleasant stimuli, especially the prick of a pin. This soreness, however, although disagreeable and hampering to the patient, cannot be considered as in any way abnormal; it is one of the phenomena of restoration of function, and occurs solely over areas where sensibility to the finer aspects of touch are considerably diminished or completely lost. Provided this excessive response to painful stimuli is confined within the limits usually assigned to the injured nerve, it is not an unfavourable sign.

The only conditions which can justly be called painful nerve injuries are those associated with *causalgia* and *painful stumps*. Causalgia was first described in 1813 by Denmark (3), who reported the case of a man wounded at the storming of Badajos. This description was followed in 1838 by a more general account by Hamilton (4), who noticed that the pain and tenderness which follow nerve injuries may be accompanied by redness and swelling so as to resemble the appearance associated with a deep collection of inflammatory matter. The first complete account was given by Paget (6) in 1864. ' In well-marked cases ', he says, ' the fingers which are affected are usually tapering, smooth, hairless, almost devoid of wrinkles, glossy, pink and ruddy, or blotched as if with permanent chilblains. They are commonly also very painful, especially on motion, and pain often extends from them up the arm.' To Weir Mitchell, Morehouse, and Keane (10) is due the credit of a complete description of a series of cases illustrating this condition. Moreover, they gave to it the name ' causalgia ', signifying a burning pain. They pointed out the intense psychical disturbance which so commonly accompanies pain of this character, and associated it particularly with injuries to nerves of the upper and lower extremities.

In some cases, if the limb has been amputated in the hope of relieving causalgia, the pain has persisted, and is then referred to the phantom limb. This brings causalgia into relation with the pain that may follow amputation, a condition usually spoken of as a ' painful stump '. It is true that painful stumps do not often cause such profound morbid manifestations as those of a case of true causalgia, but the pair belongs essentially to the same physiological class. It is characterized both by its persistence and paroxysmal exacerbations. It may be provoked not only by direct stimuli, such

as handling the affected part, but even by the fear of contact. The pain may be referred to the whole area to which the nerve is distributed; that is to say, beyond the parts which would become insensitive to light touch after complete division of the nerve.

In all cases of pain of this class there are two factors: the first is a local abnormality of the nerve associated with continuous painful impressions, and the second is the constitutional and temperamental reaction of the patient to pain.

The results produced by afferent impulses depend, not only on the strength of the stimuli giving rise to them, but also on the automatic resistance of the individual to painful impressions. Wherever pain is a prominent feature of a morbid state, it is important not only to determine the condition of the nerve, but to study day by day the reaction of the patient. He must be given some understanding of the condition from which he suffers, and of the difficulties to be overcome, so that he may realize the necessity for his wholehearted co-operation. He must share the responsibilities of treatment, and must not be promised a cure from the action of some infallible operation or drug. He must not be allowed to indulge his natural tendency to rely helplessly on the physician or surgeon. This attitude in itself would tend to prolong his morbid state and to give rise to despair if relief is delayed.

Operative treatment is frequently valuable and sometimes necessary; but if it is to have a fair chance of success it must be undertaken early, before the patient's resistance is broken. In a demoralized patient even division of the posterior roots may not be successful in removing the pain. And, moreover, it must be remembered that an operation which has miscarried will leave him worse off than he was before.

It should be recognized that in the majority of cases of causalgia pain tends to decrease and ultimately to disappear. This expectation must be explained to the patient, and he must be made aware that any operation that may be performed is intended to facilitate recovery and not of necessity to give immediate and permanent relief. Removal of accessory causes of worry, and the gradual arousal of the patient's interest in some occupation, are invaluable parts of the treatment.

But it must be remembered that, in consequence of loss of sleep and constant pain, most of these patients are in a state of exhaustion and lowered vitality. This necessitates careful regulation of the patient's activities and insistence on a sufficient amount of rest.

PART II. SPECIAL NERVES

There are many points in the diagnosis and treatment of nerve injuries which are common to all peripheral nerves, but there are special points in connexion with each individual nerve which require special mention and will now be discussed.

It is intended here neither to enter upon a discussion of the many academic and scientific questions which are associated with the physiology and pathology of the peripheral nervous system, nor to lay down any hard and fast rules in regard to the procedure which

should be adopted in the treatment of nerve injuries, but to collect and propagate the knowledge which has been gained by a number of observers as the result of their experience during the war.

The choice of the musculo-spiral nerve as the first to be considered has been influenced by the facts (1) that it is one of the commonest to be injured, (2) that it is mainly a motor nerve and therefore suffices to illustrate the principles of treatment which are applicable to other motor nerves.

1. Musculo-spiral Nerve.

A. *Diagnosis.*

The muscles supplied by the musculo-spiral nerve in the order of their branches are the long, inner and outer heads of triceps, anconeus, supinator longus, extensor carpi radialis longior, extensor carpi radialis brevior, supinator brevis, extensor communis digitorum, extensor minimi digiti, extensor carpi ulnaris, the three extensors of the thumb, and extensor indicis.

The best method of testing the function of triceps is to allow the patient's arm to hang over the back of a chair with the upper arm horizontal and the forearm hanging loosely at a right angle to it. The patient is then asked to straighten his arm (Fig. 2). By this method the muscle can be both inspected and palpated during the attempt.

Supinator longus is brought into action when flexion at the elbow is attempted with the forearm semi-pronated. A convenient test is the following: the patient is asked to clasp his hands together in front of him, with the arms slightly flexed at the elbow, and the examiner resists further flexion. Supinator longus in the unaffected limb stands out prominently, and any asymmetry will be readily observed (Fig. 3).

Weakness of extensors of the wrist can be observed when the patient attempts to raise the hand against the action of gravity, but can also be demonstrated when the patient is asked to grasp some object firmly in his hand. Normally, flexion of the fingers is associated with synergic contraction of the extensors of the wrist, and when the latter are paralysed, the effect of grasping brings about flexion of the wrist as well as of the fingers (Fig. 4). For this reason palpation of the extensor muscles in the forearm, when the fingers are being strongly flexed, enables the observer to detect any contraction in the extensor muscles of the wrist, if the latter can be brought into action. This may occur before direct voluntary action is observed.

It should be remembered that the long extensors of the fingers extend the latter only at the metacarpo-phalangeal joints, and that when these muscles are paralysed the patient, in his attempt to extend, brings the interossei into action and thus flexes his fingers at the metacarpo-phalangeal joints, and extends them only at the inter-phalangeal joints.

In injuries of the musculo-spiral nerve special attention should be paid to the patient's power of extending his thumb at the



Fig. 2. Illustrating the position of the arm preparatory to testing the action of triceps.

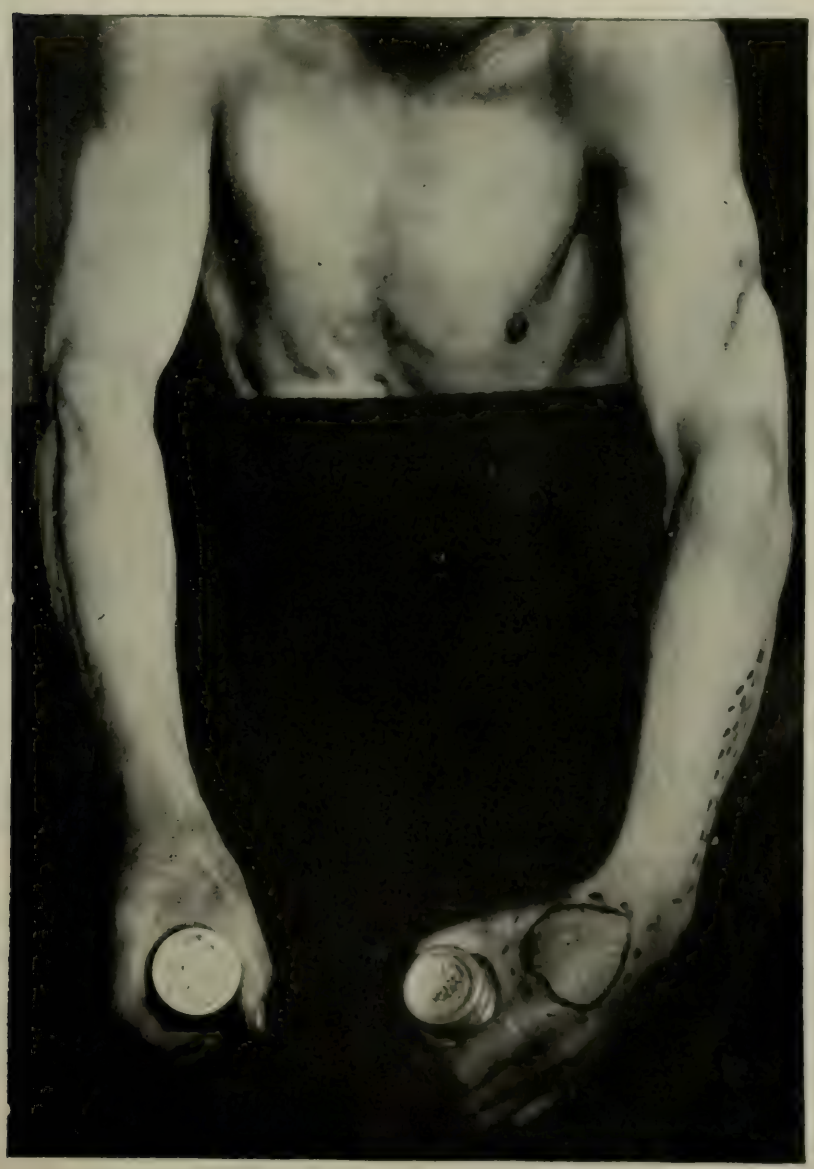


Fig. 4. To show loss of synergic action of the extensors of the wrist after division of the musculo-spiral nerves in the left upper arm. (From Dr. Grainger Stewart and Mr. Rowley Bristow's article in Sir Robert Jones' *Orthopædic Surgery*.)



Fig. 3. Division of the left musculo-spiral nerve in the upper arm. To show the asymmetry of the forearms from paralysis of the left supinator longus when the patient bends his elbows. (From Dr. Grainger Stewart and Mr. Rowley Bristow's article in Sir Robert Jones' *Orthopædic Surgery.*)

carpo-metacarpal and the metacarpo-phalangeal joints. In the case of the terminal phalanx extension is often performed in complete musculo-spiral palsy by the contraction of certain intrinsic hand muscles. These muscles can be felt to contract when the first interosseous space and the thenar eminence are palpated, while extension of the terminal phalanx is being carried out.

Demonstrable sensory changes in the hand may, on rare occasions, be absent even when there is a complete lesion of the musculo-spiral nerve at any level.

B. *Level of Lesion.*

(*a*) If the functions of the circumflex or subscapular nerves are affected together with those of the musculo-spiral nerve, the lesion involves the posterior cord of the brachial plexus.

(*b*) With a wound in the neighbourhood of the middle of the humerus the paralysis of the triceps may be partial only, although the musculo-spiral nerve is completely divided. This is due to the fact that branches to the long and inner head of the triceps come off at a higher level, and may escape when lower branches to the inner and outer heads are involved.

(*c*) Owing to the fact that the branches to supinator longus, and less frequently to extensor carpi radialis longior, arise from the nerve about the middle of the arm, these muscles may escape paralysis in the case of a lesion of the musculo-spiral nerve in the lower fourth of the arm, in the neighbourhood of the elbow joint, or when the lesion is associated with fracture of the external epicondyle of the humerus. When the wrist is extended by extensor carpi radialis longior only, the movement is characterized by some radial deflexion of the hand.

(*d*) Although the lesion may involve the posterior interosseous nerve at the point where the musculo-spiral nerve divides, extensor carpi radialis longior escapes paralysis owing to its supply coming off the musculo-spiral nerve trunk. Occasionally extensor carpi radialis brevior is also supplied from the trunk.

(*e*) The higher the lesion of the musculo-spiral nerve the more likely are there to be sensory changes over the posterior aspect of the first interosseous space (Fig. 5). No gross sensory changes are to be expected from division of the radial nerve alone.

C. *Treatment.*

Observation stage.—When a medical man is confronted with a peripheral nerve lesion he will require some data upon which to form an opinion as to whether operative intervention is or is not required. In some cases these data, based upon previous reliable observations, may be forthcoming, but very often the only information will be the date of the injury and the patient's own account of subsequent operations and of the evolution of his symptoms.

The best procedure to adopt will naturally vary with the history of the case, and from this point of view cases may be grouped under the following headings.

(1) When there is no evidence that an operation has been performed on the nerve.

(*a*) The paralysed muscles have received efficient treatment (relaxation, massage, and electricity), and there is no sign of recovery two months after the date of the injury. In this case there is no reason to delay exploration of the nerve.

(*b*) The paralysed muscles have not received appropriate treatment. In this case absence of improvement does not necessarily indicate a failure of recovery on the part of the nerve, and the patient should be kept under observation for a period of two months, during which relaxation should be instituted and daily treatment carried out.

(2) Where there is trustworthy evidence that the nerve has already been explored and left in a condition favourable to recovery.

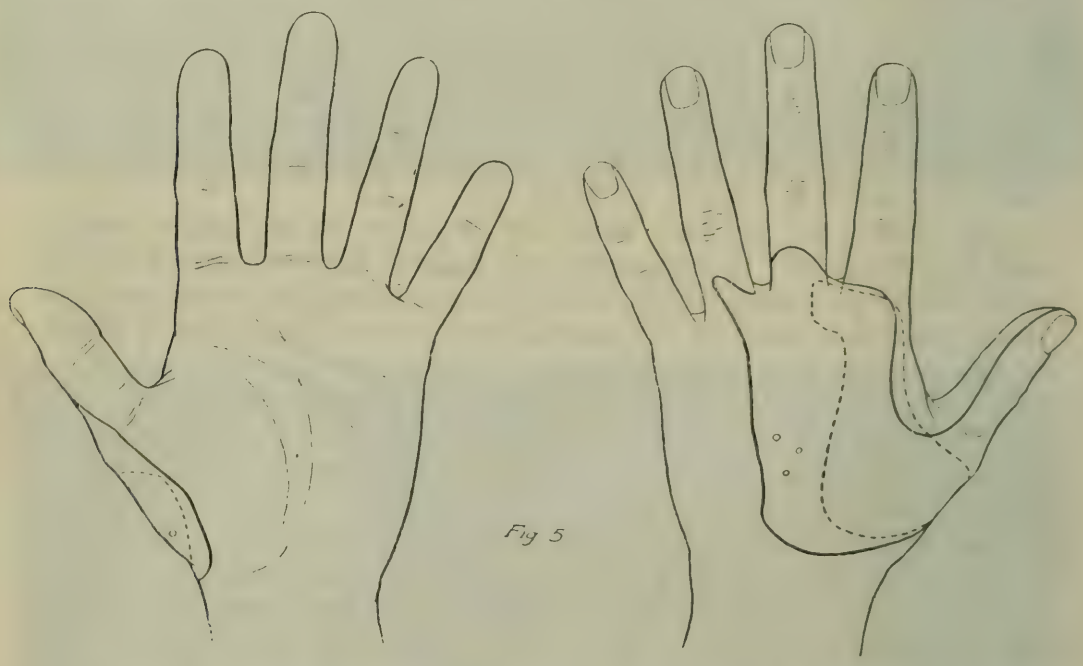


FIG. 5. To represent the area of loss of cutaneous sensibility after complete division of the musculo-spiral nerve by a bullet high up in the axilla. The area of insensibility to light touch enclosed by the interrupted line is considerably smaller than that to pin-prick. The small rings indicate the position of six spots where pin-pricks were appreciated. Deep sensibility was nowhere abolished.

In such a case it is evident that the observation stage must last a great deal longer than two months before the question of a further operation need be considered. The exact length of the waiting period will depend on the nature of the operation which was carried out.

The treatment during the period of observation can be divided into passive and active measures.

Treatment by splints must be so arranged that there is fixed extension of the wrist associated with elastic extension of the fingers at the metacarpo-phalangeal joints, and special attention should be paid to the thumb, which must be kept in a position of abduction. A splint taking purchase on the palm of the hand should not extend beyond the proximal transverse crease and should not be broad

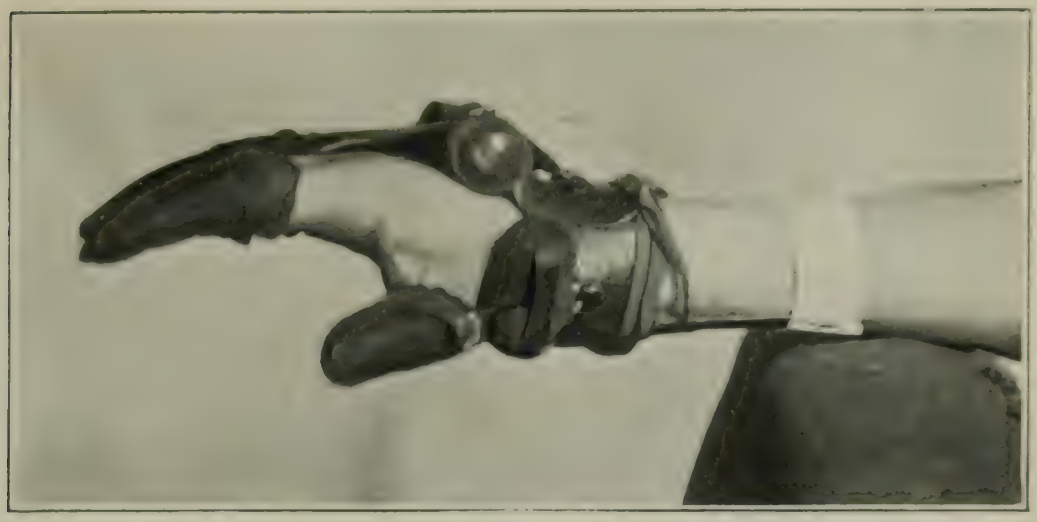


Fig. 6 *a*. Elastic extension apparatus for musculo-spiral palsy.
Hand at rest; side view.



Fig. 6 *b*. Elastic extension apparatus for musculo-spiral palsy.
Hand at rest; front view.

[Fig. 6 *a*, *b*, *c*, *d*. A useful form of elastic extension apparatus for the hand, fingers, and thumb, in cases of musculo-spiral palsy.]



Fig. 6 *c*. Elastic extension apparatus for musculo-spiral palsy. Hand clenched; side view.

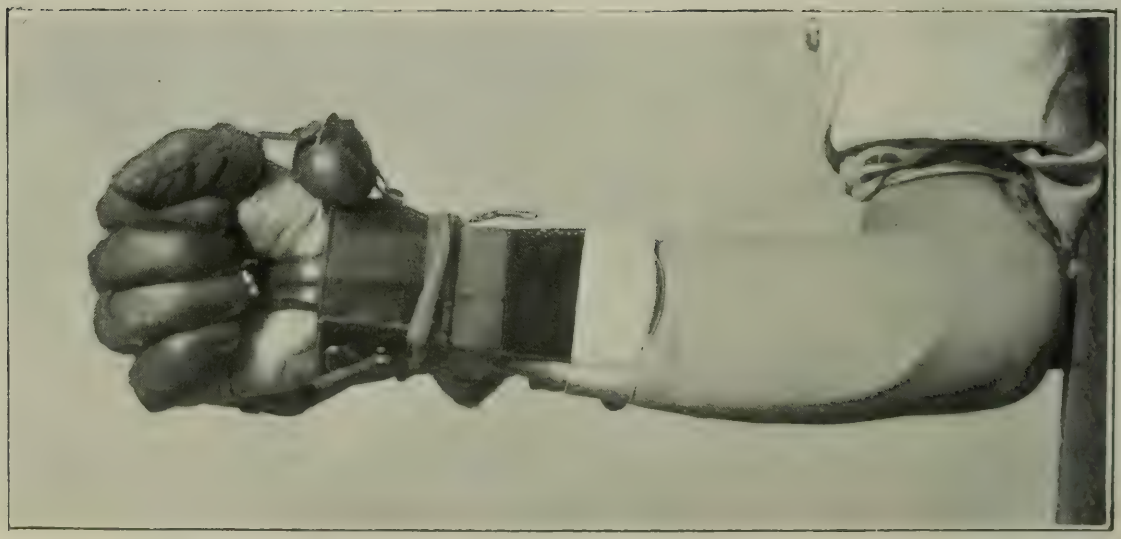


Fig. 6 *d*. Elastic extension apparatus for musculo-spiral palsy. Hand clenched; front view.

[Fig. 6 (*a*, *b*, *c*, *d*). A useful form of elastic extension apparatus for the hand, fingers, and thumb, in cases of musculo-spiral palsy.]

enough to press on the thenar and hypothenar eminences. There is little danger of the wrist becoming permanently fixed in a position of extension, but there is great danger of obstinate ankylosis of the metacarpo-phalangeal joints if these are fixed rigidly. For this reason an elastic apparatus, which allows voluntary flexion at these joints, and at the same time brings the fingers back to a position of moderate extension during rest, is valuable (Fig. 6).

No special precautions need be taken for the purpose of relaxing triceps and supinator longus.

The combination of massage and electricity is the ideal treatment, but when only one form of treatment is available massage is to be preferred, especially in cases where arthritic changes have developed. The form of electricity to be used is that current which will make the muscles contract, and this is the interrupted galvanic current in the large majority of cases. The current should be of sufficient strength only to make the muscles contract, and certainly not strong enough to spread to the antagonistic muscles so that they are brought into action. For this reason it is advisable to continue with the galvanic current in the treatment of a recovering muscle until such time as it will respond to a faradic current which does not cause contraction of the antagonists.

As soon as the muscles show any evidence of returning function a certain amount of time should be devoted during each session of treatment by the masseur in encouraging the patient to make active movements. It is important that the limb should be protected from cold and from the pressure of badly fitting splints interfering with the circulation in the affected muscles.

The trick movements described on p. 6 are particularly to be guarded against.

Indications for operation.—If at the end of a period of two months of observation and treatment on the lines indicated above there is no evidence of returning function, an exploratory operation should be undertaken. This rule holds good for uncomplicated cases, but may need qualifying by the following considerations.

(1) No operation, undertaken with the idea of suturing the nerve if found to be divided, should be carried out until the wound has been soundly healed for at least a month. This interval between the healing of the wound and the operation may need to be much prolonged in the case of wounds complicated by septic compound fractures.

(2) When the nerve lesion is associated with an ununited fracture necessitating an operation on the bone, it is generally unwise to attempt the union of the bone and of the nerve at the same time. If the latter is carried out first, it is advisable to wait at least a month before operating on the bone, in order that the sheath of the nerve may be firmly healed, and thus form a protective barrier against possible sepsis lighted up by the second operation.

(3) An intact humerus should not be shortened in order to bring the nerve ends together. But if an operation for ununited fracture necessitates shortening of the humerus, this should be carried out before any attempt is made to suture the nerve.

(4) Delay in uniting the ends of a divided nerve does not seriously

affect the prospects of success. As far as is known, there is no interval of time between the division and union of the nerve long enough to exclude the possibility of recovery.

In the case of the musculo-spiral nerve, evidence of returning function depends on the recovery of voluntary power and on the presence of a response in the muscles to the faradic current. Sensory and trophic changes cannot be relied upon, and the quality of the galvanic response is too much influenced by the condition of the muscles, subcutaneous tissue, and skin to be a sign of much value.

Operation.—The incision should be long enough to enable the surgeon to identify the nerve above and below the site of injury. Care should be taken that the nerve is not roughly handled, especially by metal instruments, and powerful traction on the proximal end should be avoided. Large muscular branches on the external aspect of the humerus must not be mistaken for the main trunk. It must be remembered that the divided ends may be deflected far from their normal position.

Facilities for applying a faradic current should be at hand, and in a few cases the results of such an examination may be of value in deciding whether resection of an incompletely divided nerve should be done. When there is complete anatomical division it is unnecessary to investigate the function of the peripheral end by electrical tests.

In considering this question of resection the surgeon may remember that the results of suturing this nerve are generally favourable and that, in the event of failure, there are other means of making the wrist and fingers functionally useful.

(1) Cases in which resection and suture should certainly be attempted :

(*a*) When there is complete absence of anatomical continuity between the proximal and peripheral ends.

(*b*) When there are two distinct bulbs.

(*c*) When there is a bulb at the site of injury occupying the whole thickness of the nerve.

(*d*) When a neuroma and scar tissue involves more than a third of the thickness of the nerve. If less than a third of the nerve is scarred a partial resection and suture may be considered.

(2) Resection should not be done when dissection reveals a nerve which is neither very hard nor attenuated and the sheath of which is intact.

(3) Doubtful cases.—The surgeon should take the following points into consideration :

(*a*) Length of time since the injury without return of function in spite of adequate treatment.

(*b*) Consistence, colour, and calibre of the nerve at the site of injury.

(*c*) The appearance and consistence of the nerve trunk peripheral to the site of injury.

(*d*) Electrical examination of the exposed nerve.

(*e*) The facility with which suture of the divided ends could be performed.

In preparing the ends for suture all scar tissue including bulbs must be removed from the proximal and peripheral ends until the bundles of nerve fibres can be clearly differentiated. These may have a swollen and gelatinous or sago-like appearance without prejudice to the success of the operation.

If there is difficulty in approximating the ends, a fixation suture passed through the substance of the nerve may be used. When this difficulty does not obtain, fine sutures through the nerve sheath only will suffice. These sutures should not be tied so tightly as to squeeze the ends together, a very narrow gap being rather an advantage than otherwise. Care should be taken to avoid axial torsion as far as possible.

The methods recommended for dealing with gaps in the nerve are three in number.

(1) Relaxation by posture. A more or less extensive freeing of the nerve combined with flexion of the elbow will usually allow the ends of the nerve to be brought together. In such a case flexion of the joint should be maintained for a period of four weeks, at the end of which time gradual extension of the elbow may be allowed.

(2) Transposition of the nerve to the front of the humerus. In this operation a new course is made for the nerve in front of the humerus, thus shortening the distance traversed by at least an inch.[1]

(3) The insertion of a graft. If this is resorted to a tendon transplantation should be done as well. For a graft a portion of an unimportant nerve, such as the internal cutaneous, from the same patient, or of a nerve from a limb which is being amputated at the same time, should be used for preference.

In all cases in which the cut ends of the divided nerve cannot be approximated, and in all cases in which recovery of function after

[1] The following description of this operation has been supplied by Sir Harold Stiles:

(*a*) Lower external dissection. This is carried out in the usual manner, and the two ends of the nerve completely freed as far as possible in both directions.

(*b*) Upper internal dissection. The incision is made along the line of the median nerve, and exposes the inner border of coraco-brachialis and biceps. The deep fascia having been divided, the median nerve is exposed and retracted inwards along with the vessels, while the edges of the muscles are freed and retracted outwards. In doing this the muscular branches passing between the muscles and the main vessels are divided between forceps. After these have been divided, the median and internal cutaneous nerves and the main vessels can be retracted further inwards and backwards so as to expose the superior profunda vessel a little below the lower border of latissimus dorsi. These vessels are isolated and separated from the musculo-spiral nerve and divided between forceps. The nerve is then exposed. At the point where the superior profunda vessels are divided it gives off two large branches to triceps. These are freed in both directions, while the main nerve is freed in an upward direction. The two muscular branches can be dissected still further upwards off the main nerve well up in the axilla, and another branch going to the long head of triceps, which is exposed in so doing, is also dissected upwards along with the others. Thus the proximal portion of the musculo-spiral is freed far up into the axilla without injuring its motor branches. The main nerve is followed downwards behind the humerus by dividing the upper fibres of the inner head of triceps. The upper stump, already freed, can now be pulled upwards into the upper dissection. A pair of curved artery forceps is then passed from the lower wound in front of the humerus obliquely upwards and inwards, until the points appear in the lower part of the upper wound. The upper stump is then seized and pulled through into the lower wound. By fully flexing the elbow the two stumps can be approximated. The upper wound must be closed before the nerve is sutured, so that the limb may be kept adducted during this procedure.

At least one inch can be gained in this manner.

suture does not occur, tendon transplantation may be depended upon to give a useful functional result. It can be carried out in the following way: pronator radii teres into extensores carpi radialis longior et brevior, flexor carpi radialis into extensores ossis metacarpi pollicis et brevis pollicis; flexor carpi ulnaris into extensores communis digitorum et longus pollicis. Suture of the tendons must be performed with the hand, fingers, and thumb in the position of full extension, and the tendons must be brought to their point of insertion by the straightest possible route.

Post-operative treatment.—The treatment of the limb after operation should be similar to that adopted during the period of observation, modified, if necessary, by surgical considerations in regard to the condition of the wound and posture of the limb.

D. *Signs of Returning Function.*

Before any alteration of function can be detected there is often a stage when the patient states that 'the hand and arm feel more life-like', or that 'the skin on the back of the hand feels different'.

Evidence of returning muscular function is perhaps more often first afforded by the detection of a faradic response than by the demonstration of a voluntary contraction, but the reverse may be the case. Before the patient is able to extend his wrist against the power of gravity he will be able to support it when it has been passively placed in the extended position. The more proximal the injury the later are the first signs of recovery. For instance, the average figures in a number of cases were as follows:

Level of lesion.	*Time in months.*
Lower third of upper arm	4–5
Middle ,, ,,	5–6
Upper ,, ,,	7–8

The prognosis for nerve suture is as good when two or more years have elapsed since the date of injury as when an early operation has been performed.

Speaking generally, the prognosis is so good after suture of the musculo-spiral nerve that the operation can always be recommended. When the ends cannot be brought together, even by bringing the nerve along the inner side of the arm, there is no justification for shortening the humerus. The results of tendon transplantation are so satisfactory when carried out by an experienced surgeon that the necessity for depending on the doubtful results of grafting the nerve does not exist.

2. Median Nerve.

A. *Diagnosis.*

Motion.—The muscles supplied by the median nerve, in the order of their branches, are pronator radii teres, flexor carpi radialis, palmaris longus, flexor sublimis digitorum, flexor longus pollicis, flexor profundus digitorum, pronator quadratus, the two radial lumbricals, opponens pollicis, abductor brevis pollicis, and the outer head of flexor brevis pollicis.

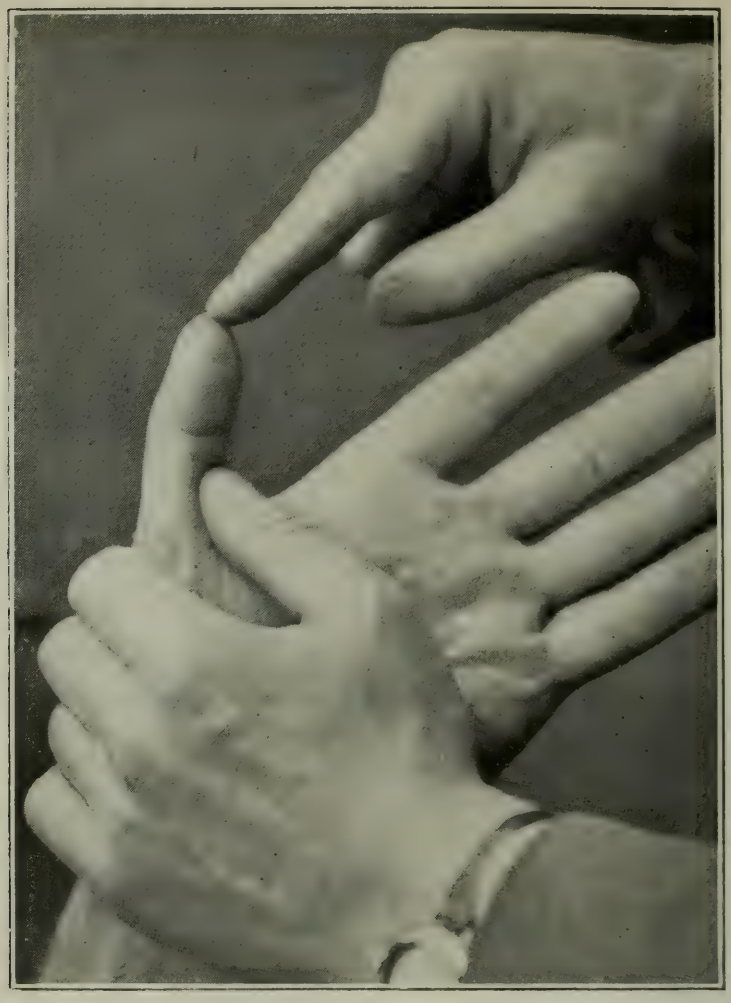


FIG. 7. To illustrate the method of testing the action of the flexor longus pollicis by ascertaining whether flexion of the terminal phalanx of the thumb can be maintained against resistance.



FIG. 8. To show the correct posture of the thumb in relation to the index finger preparatory to testing for voluntary contraction in abductor brevis pollicis.

The nerve to the superficial flexor of the index finger comes off the median in the lower third of the forearm, whereas the branches to the superficial flexors of the middle, ring, and little fingers are given off higher up in the forearm. The deep flexor of the middle finger is innervated both by the ulnar and the median nerve.

In testing for voluntary action in pronator radii teres, the patient should be asked to pronate the forearm from the position midway between pronation and supination.

It must be remembered that the wrist can be flexed by abductor longus pollicis (extensor ossis metacarpi pollicis) or by muscles supplied by the ulnar nerve as well as by those supplied by the median. It is important, therefore, to make sure that the median flexors are in action when this movement is being carried out.

A simple method of making certain whether the flexors of the fingers are paralysed or not is to fix the patient's hand at the wrist and metacarpo-phalangeal joints, and to passively flex his fingers. The patient is asked to resist an attempt made to straighten the fingers. It should be remembered that the interossei flex the fingers at the metacarpo-phalangeal joints.

Flexor longus pollicis flexes the terminal phalanx of the thumb. Slight flexion of the terminal phalanx of the thumb does not necessarily mean that this muscle is in action unless it can be carried out against resistance (Fig. 7).

Abductor brevis pollicis is one of the most difficult muscles to test for voluntary action, for abductor longus pollicis (extensor ossis metacarpi pollicis), supplied by the musculo-spiral nerve, abducts and extends the thumb. The following method of testing the action of abductor brevis pollicis obviates this difficulty. Place a pencil across the palmar surface of the extended fingers, and let the patient hold it in that position by fixing it between the straightened thumb and the index finger (Fig. 8). Then ask him to separate his thumb from his finger in a plane at right angles to the palm. If abductor brevis pollicis is acting he should be able to release the pencil without extending his thumb.

The presence or absence of opposition of the thumb was at one time thought to be of great diagnostic importance with regard to lesions of the median nerve. Experience has shown that this is not the case and that good opposition can be brought about by other muscles than opponens pollicis.

Wasting of the muscles of the thenar eminence, so that the outer edge of the metacarpal bone can be seen and felt, is the best evidence that the intrinsic muscles innervated by the median nerve are deprived of their nerve supply.

Sensation.—If a patient complains of pain within the median area of the hand, this is no valid reason for believing that the lesion is not complete.

Retention of deep sensibility does not necessarily mean that the lesion is incomplete. Pressure can be appreciated and localized in any part of the median distribution in the hand, when the nerve has been divided in the neighbourhood of the wrist, provided the long flexor muscles of the fingers and their tendons are uninjured. On the other hand, complete lesions of the median nerve above the

elbow are accompanied by loss of deep sensibility in the termina phalanges of the index and middle fingers.

With division of the median nerve loss of appreciation of pin-prick may be confined to the terminal phalanges of the index and middle fingers, the area of loss being somewhat more extensive on their palmar than on their dorsal aspect.

When the median nerve is divided, the area over which light touch cannot be appreciated is fairly constant. It is usually bounded by a line running from the radial edge of the thumb-nail, along the radial border of the thumb, to the fold at the base of the thenar

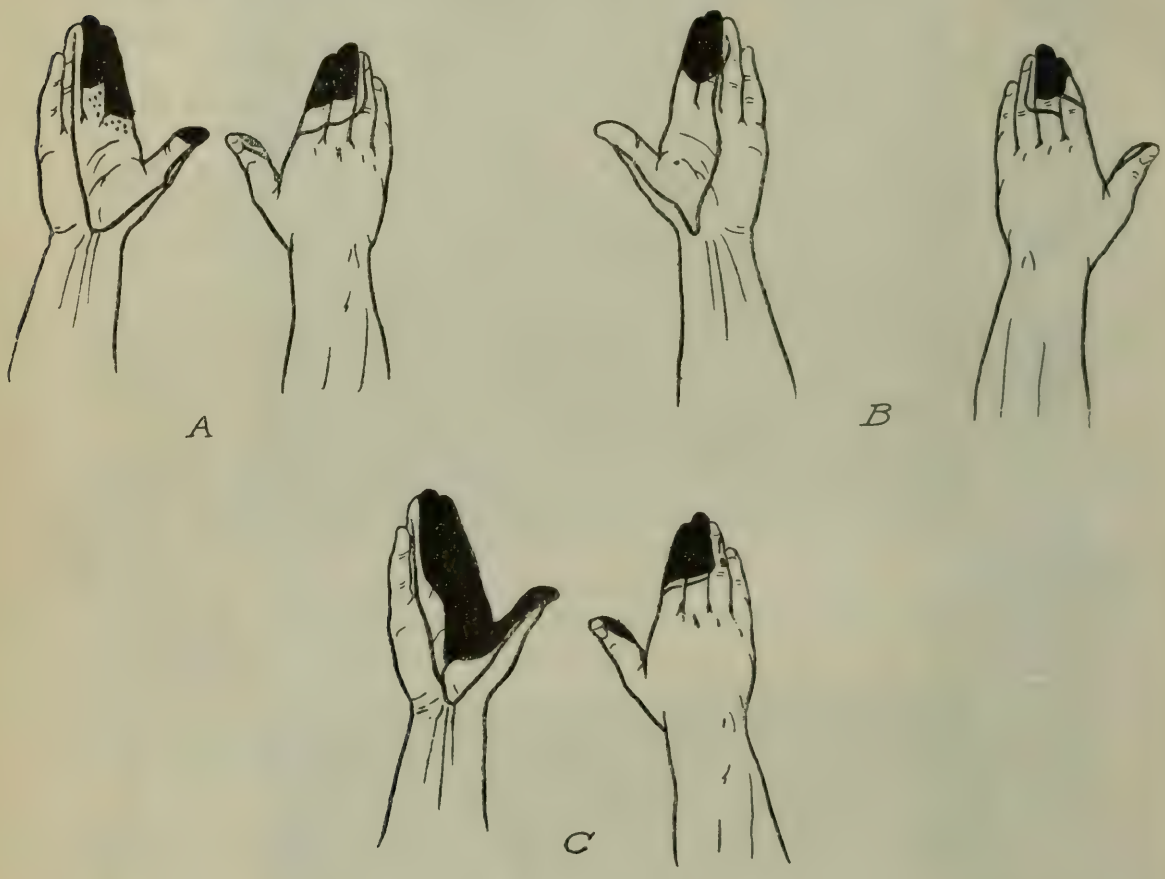


Fig. 9. To show average (A), small (B), and large (C) areas of cutaneous sensory loss after division of the median nerve. The areas of complete analgesia are coloured black and those of insensibility to light touch are enclosed by continuous lines. (After Head and Sherren.)

eminence. From the inner border of the thenar eminence it passes up the palm, more or less directly, to the cleft between the middle and ring fingers. It includes a variable portion of the radial half of the palmar surface of the ring finger, and on the dorsal surface the radial third of the terminal two phalanges of the ring finger, and the skin over the terminal two and a half phalanges of the middle and index fingers. From the radial side of the index finger the border runs along the free edge of the fold of the first interosseous space, and extends up the thumb to end at the ulnar border of the nail (Fig. 9).

B. *Level of Lesion.*

(*a*) A lesion of the median nerve in the middle of the forearm may paralyse the superficial flexor of the index finger, while allowing those of the other three fingers to escape.

(*b*) With complete division of the median nerve above the point of origin of the branches to the muscles of the forearm, deep sensibility is lost in the terminal phalanx of the index finger. The highest motor branch is that to pronator radii teres, which is given off in the ante-cubital fossa.

(*c*) If the median nerve is severed in the neighbourhood of the wrist without division of the flexor tendons, deep sensibility is retained in the last phalanx of the index finger (except perhaps in its tip).

(*d*) The palmar muscular branch arises at the level of the trapezoid ridge or the lower edge of the annular ligament.

(*e*) The point of origin of the anterior interosseous nerve is very variable. Hence evidence of injury of this nerve is of little value in regional diagnosis.

C. *Vasomotor and Trophic Changes.*

These changes in complete lesions of the median nerve may be confined to the index and middle fingers. The palmar aspects of these fingers do not sweat, and they are liable to suffer from burns and other injuries. Blisters and ulcers on the first and second fingers are other manifestations of the disordered nutrition. Many of the other changes which are often regarded as due to damage to the nerve are really the outcome of defective nutrition brought on by disuse and immobilization of the hand by splinting. It is not sufficiently recognized that the soft tissues of the wrist afford poor protection to the vessels and nerves supplying the hand. Consequently relatively light pressure at the wrist (the pedicle of the hand) may lead to gross nutritional changes in the hand and fingers.

D. *Differentiation between Complete and Incomplete Lesions.*

Certain clinical indications help to differentiate incomplete lesions of the nerve from those in which solution of continuity is complete. When the lesion is incomplete sensation returns earlier, the borders of the areas of loss to pin-prick and light touch retreat concomitantly as recovery goes on. The affected muscles retain their nutrition better, and motor power is recovered more quickly.

The lesion is incomplete if the hand sweats and there are gross changes in the finger joints.

E. *Treatment.*

Observation stage.—The general principles of treatment by passive and active measures during the stage of observation, and the general indications for operation with lesions of the median are those already laid down on p. 16 for nerve injuries in general. Splinting is unnecessary.

Indications for operation.—There are no special indications for operation on the median nerve except in so far as that the frequency

of persistent pain not uncommonly renders necessary exploration of a nerve manifestly not divided.

Operation.—The only mechanical difficulties encountered in exposure of the nerve are found in the upper part of the forearm. Here pronator radii teres crosses and restricts the field of operation. It is advisable to divide and reflect the muscle as soon as it has been exposed.

When operating on incomplete lesions of the median nerve, the surgeon should bear in mind the importance of its sensory supply, and should never sacrifice this for the purpose of obtaining a better motor function. Conversely, the disability due to the total sensory loss is almost equivalent to that resulting from a complete division of the nerve. The surgeon may justifiably sacrifice existing partial motor function in the hope that complete excision of the damaged part and suture may result in a satisfactory return of sensibility.

An incomplete sensory recovery may in itself be very disabling.

In cases of injury of the median nerve complicated by causalgia, if the sensory loss is not great the nerve should be injected with alcohol above the lesion for relief of the pain, unless other data indicate clearly that the damaged portion should be excised. But if the pain is associated with gross loss of function, and especially with extensive analgesia, there need be no hesitation in deciding to resect the nerve.

In the forearm a gap in the nerve measuring two inches or less can be dealt with by flexion of the fingers, hand, and forearm. With a gap of more than two inches in length a two-stage operation is advised. At the first stage, with the fingers, hand, and forearm in extreme flexion, the bulbs are drawn as near together as possible by tying a ligature round them. The wound is then closed and allowed to heal. At the end of a week the arm may be released from its position of strong flexion, and by the end of about three weeks the patient is ready for the second stage of the operation. The nerve is exposed again and the ends are sutured together in the ordinary way, with the segments of the arm flexed. At the first stage of the operation no attempt is made to freshen the ends of the nerve, this being done in the second operation.

In the upper arm the two-stage operation can be employed to overcome a gap of two and a half inches or more, the ends being approximated by adduction of the arm at the shoulder and flexion of the elbow.

A few cases may be met with where the gap is so great that grafting is the only resource.

Of the numerous other methods which have been suggested for dealing with large gaps none has proved by experience to be successful. Shortening of the humerus or of the bones of the forearm is to be condemned. There is no form of tendon transplantation which is capable of giving a satisfactory result, for in any case it would leave the patient with a hand seriously disabled through anaesthesia.

F. *Signs of Returning Function.*

Return of function usually begins with recovery of sensibility. This precedes any return of voluntary power to the muscles or restoration of faradic response.



Fig. 10. Division of the ulnar nerve in the forearm below the origin of the branches to flexor carpi ulnaris. To show the tendon of flexor carpi ulnaris standing out when the patient adducts his hand.

After suture of the nerve the time required for return of voluntary muscular contraction depends on the level of the nerve lesion, and on the distance which has to be traversed by the regenerating fibres as regards individual muscles.[1]

3. Ulnar Nerve.

A. *Diagnosis.*

Motion.—Muscular branches are given off by the ulnar nerve in the following order: to flexor carpi ulnaris and the inner half of flexor profundus digitorum near the elbow; to palmaris brevis just below the wrist; to all the muscles of the hypothenar eminence, the two inner lumbricals, all the palmar and dorsal interossei, adductor pollicis, and the inner head of flexor brevis pollicis.

There are certain difficulties in testing the functions of some of these muscles. It has, for example, to be remembered that the extensors normally abduct, and the flexors adduct the fingers as a whole, so that the action of the interossei, in carrying out the lateral movements of the fingers, can be tested only when the long flexors and extensors are prevented from acting. For this reason the hand should be laid on the table and kept in contact with it during the test. For instance, when testing the action of abductor minimi digiti, if the finger rises from the table abduction is being performed by the long extensor. Should the little finger be in the clawed position this test cannot be carried out, and the finger should then be placed in a position of slight flexion at the metacarpo-phalangeal joint before the test for abduction is attempted.

The first dorsal interosseous may occasionally be supplied by the median nerve.

The following method of testing the function of flexor carpi ulnaris has been found useful. The patient closes his hand and the observor then adducts it strongly, placing his finger on the tendon of flexor carpi ulnaris. When the patient opens his hand the tendon will be felt to tighten if the muscle is in action (Fig. 10).

The reaction of this muscle to the faradic current is particularly easy to test, and may be of great diagnostic value in doubtful cases.

The action of the first dorsal interosseous is difficult to test, but it can be examined by the following method. The thumb is placed in

[1] The following data on functional recovery after suture of the median nerve are taken from Professor Stopford's statistics:

1. Lower third of forearm and wrist (14 cases):
   Sensibility to pin-prick, 3–4 months.
   Abductor brevis pollicis recovered in 5 out of 14 cases.
2. At the bend of the elbow, above the origin of the motor branches (10 cases):
   Pronator radii teres, Flexor carpi radialis } 4 months.
   Abductor brevis pollicis recovered in 3 out of 10 cases.
3. Upper arm (8 cases):
   Pronator radii teres, Flexor carpi radialis, Palmaris longus } 6 months.
   Abductor brevis pollicis recovered in 3 out of 9 cases.

Sensation in the index finger is last to recover, and after even a long period is often imperfect.

a position of abduction, and the observer palpates the tissues of the first interosseous space. At the same time the patient attempts to extend the terminal phalanx of his thumb against resistance. A normal first dorsal interosseous muscle can, under these conditions, be felt to contract.

Sensation.—Painful sensations may be referred to the hand though the section of the ulnar nerve is complete; these are due to mechanical stimulation of a bulb on the proximal end of the nerve.

Loss of deep sensibility is limited, as a rule, to the little finger,

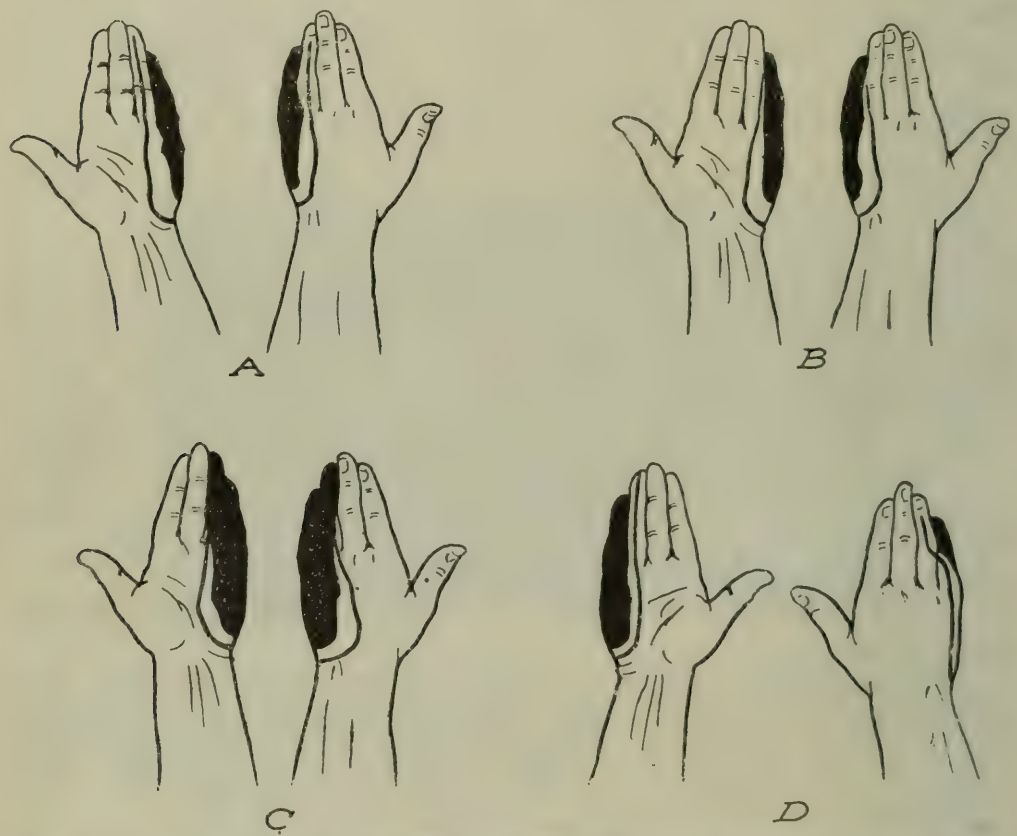


FIG. 11. To illustrate the loss of cutaneous sensibility produced by division of the ulnar nerve. The area representing the loss to light touch is surrounded by a continuous line, while that of analgesia is shaded black. A represents an average, B a small, and C a large area of sensory loss. Figure D (after Head and Sherren) indicates the extent of cutaneous anaesthesia and analgesia after complete section of the ulnar nerve below the origin of the dorsal branch.

and is present only when the lesion is as high as the upper part of the forearm. When, however, the tendons of flexor profundus digitorum are divided at the wrist along with the nerve, the area of loss is the same as with complete division of the nerve at the elbow.

Usually the area of insensibility to pin-prick covers the little finger and the ulnar border of the hand, but it is subject to great variation. When the nerve is divided at the wrist the dorsal branch may escape. Preservation of sensibility on the dorsal aspect of the hand, even when the lesion is as high as the elbow, does not necessarily mean that the injured nerve has escaped complete division. It may be due to escape of the dorsal branch owing to an unusually high origin.

The usual extent of loss to light touch (cotton-wool) after complete division of the ulnar nerve is an area comprising the little finger, the ulnar half of the ring finger, and that part of the hand which is limited, on the dorsal and palmar aspects, by a line continuous with the axis of the ring finger. Eighty per cent. of Professor Stopford's cases showed this distribution.

The extent of this area of loss to light touch is more constant than that of the loss to pin-prick (Fig. 11).

B. *Level of Lesion.*

The branch to flexor carpi ulnaris is given off usually about 1 in. below the internal condyle of the humerus. There are often two branches a short distance apart, one larger than the other.

As already mentioned, sensibility may remain undisturbed on the dorsal aspect of the hand owing to a high origin of the dorsal branch, though division of the nerve is complete at a point as high as the elbow.

C. *Vasomotor and Trophic Changes.*

After complete division of the ulnar nerve the little finger and a variable portion of the ulnar border of the hand do not sweat. Burns are of frequent occurrence on the little finger, as patients are apt to rest the hand on a hot surface.

D. *Differentiation between Complete and Incomplete Lesions.*

This differentiation follows the same principles as those already described for the median nerve. Causalgia is less often associated with injury to the ulnar than to the median nerve; its presence, however, is evidence that the lesion is incomplete.

E. *Treatment.*

Observation stage.—The general rules already laid down for treatment at this stage should be followed. No special indications for operation need be added to those already given.

Operation.—The motor supply of the ulnar nerve is of paramount importance, and the functional results after suture are not so uniformly good as with the musculo-spiral. It is therefore all the more necessary carefully to consider the condition of the nerve found at operation, and to compare it with the clinical findings before deciding on the course to be followed.

It is never necessary to use a graft to overcome a gap in this nerve. As a rule the ends can be brought together at one operation. The following methods for overcoming shortening are advised:

1. Relaxation by posture and freeing the nerve.

2. For a larger gap, free the branches to flexor carpi ulnaris and flexor profundus digitorum, and displace the nerve in front of the condyle. To supply a bed for the nerve, it will be necessary either to form a tunnel in the muscles, or to dissect them from the condyle. The arm should be put up in the position of flexion at wrist and elbow and adduction at shoulder.

3. For a still larger gap it will be found best to sacrifice the

branches to flexor carpi ulnaris and flexor profundus digitorum, and to move the nerve into the middle line of the forearm. Gaps as wide as 5 in. or more in length can be overcome by freeing the nerve from axilla to wrist, and by bringing it in front of the arm and forearm.

The surgeon should never shorten the bones of the forearm or upper arm to overcome a gap in the ulnar nerve.

The operative treatment for injuries of the ulnar nerve below the wrist is a matter of great difficulty. In the neighbourhood of the pisiform bone it rapidly breaks up into its terminal branches, and an operation at this level is rarely successful in bringing the ends together.

F. *Signs of Returning Function.*

Sensibility begins to recover before motion, except with high lesions, when flexor carpi ulnaris and flexor profundus digitorum may first show signs of recovery. A better functional recovery is to be expected in flexor carpi ulnaris, flexor profundus digitorum, and in abductor minimi digiti than in the other muscles supplied by the nerve.

4. Division of both Median and Ulnar Nerves.

Wounds of the wrist are not uncommonly associated with division of both median and ulnar nerves. This leads to complete paralysis of all the intrinsic muscles and to the development of a characteristic deformity. The hand is flattened by the wasting of the thenar and hypothenar eminences, and the thumb is rotated so that its palmar aspect looks forward (Fig. 12). To prevent this deformity the thumb must be kept abducted by splinting.

At this level the results of suture may be satisfactory, but when the lesion is above the elbow, and especially when it is accompanied by injury to the brachial artery, the results of operation are very disappointing. This want of recovery is due to fibrosis of the muscles and ankylosis of the joints which so frequently follow a lesion in this situation.

5. Musculo-Cutaneous Nerve.

A. *Diagnosis.*

Motion.—In its course through the arm the musculo-cutaneous nerve supplies muscular branches in the following order: to coraco-brachialis near to the origin of the nerve, to biceps after piercing coraco-brachialis, and lastly to brachialis anticus.

Biceps supinates as well as flexes the forearm, while brachialis anticus is a pure flexor. The activity of coraco-brachialis can best be tested by its response to the faradic current.

Sensation.—The sensory distribution of the cutaneous branches on the radial side of the forearm is very variable. After division of the nerve the area of loss of sensibility to light touch and to pin-prick on the radial border of the forearm are ill defined, and deep sensibility is, as a rule, not affected.



Fig. 12 *a*



Fig. 12 *b*

Fig. 12. To illustrate the deformity of the hand and the areas of insensibility to light touch (dotted line) and pin-prick (continuous line) which followed section of the median and ulnar nerves. (From Dr. Grainger Stewart and Mr. Rowley Bristow's article in Sir Robert Jones' *Orthopaedic Surgery*.)



FIG. 13. A light adjustable abduction splint to relax deltoid in cases in which this muscle is paralysed.
To show the splint in position.

B. *Level of Lesion.*

Lesions of this nerve are usually close to its origin from the outer cord of the brachial plexus, and are often associated with injury to the median and musculo-spiral nerves.

C. *Signs of Returning Function.*

The results of suture are excellent, as might be expected from the shortness of the nerve. Voluntary contraction of biceps may be obtained in four months after nerve-suture, and, as a rule, precedes any demonstrable alteration in sensation.

6. Circumflex Nerve.

A. *Diagnosis.*

The muscles supplied by this nerve are teres minor and deltoid. It should be remembered that the arm can be abducted to some extent even although deltoid is paralysed. In order to be certain that deltoid is in action the muscle should be palpated when the attempt to abduct the arm is being made.

An oval area on the outer aspect of the upper arm in the neighbourhood of the insertion of deltoid, which varies in size in different patients, can be mapped out in which light touch and to a less extent pin-prick are not appreciated.

Damage to the circumflex nerve is usually accompanied by a lesion of the brachial plexus or fracture of the neck of the humerus.

B. *Treatment.*

This nerve is rarely completely divided. Recovery of function in deltoid depends almost entirely on keeping the muscle relaxed. Continuous abduction of the shoulder by means of some such splint as the one illustrated is essential (Fig. 13).

The presence of injury to the circumflex nerve influences the treatment to be adopted for the shoulder joint, in cases of destruction of the head of the humerus. If deltoid is permanently paralysed, the shoulder should be fixed by arthrodesis in a position of abduction at an angle of 75 degrees, and flexion of 20 degrees to the coronal plane. As, however, sepsis in the bone, if present, usually lasts for a long period, there is plenty of time in most cases to observe whether the muscle is going to recover or not before any such operation must be carried out on the joint.

7. Brachial Plexus.

Lesions of the roots, trunks, and cords which comprise the brachial plexus have been of common occurrence in the war, often complicated by injury of the spinal cord, the apex of the lung, or the great vessels at the root of the neck. Isolated involvement of a single root or cord is rarely found, and, as scar formation usually plays a large part in producing the functional defect, the clinical picture presented may vary greatly.

Treatment.

It is the rule to find recovery beginning within a few weeks of the injury, and no operation should be advised till such recovery has ceased.

The treatment adopted during the observation stage should as far as possible be that of the particular nerves involved, due prominence being given to those which are most important. When the lesion is extensive, the arm should be kept abducted at the shoulder, flexed at the elbow, and extended at the wrist. It is not necessary to make the patient wear an abduction splint for more than a year in the hope that functional recovery of deltoid will result.

When all progress has ceased, exploration may be advisable in a certain number of cases. Good results are often obtained from freeing the nerves from scar tissue.

Where individual roots are injured, and no recovery has taken place within nine months, it is advisable to explore, and excellent functional recovery has followed suture in certain cases, notably with the fifth and sixth cervical nerves.

8. Sciatic Nerve.

A. *Diagnosis.*

Motion.—From the surgical point of view the internal and external popliteal nerves, bound together by connective tissue only, are separable up to the lumbo-sacral plexus. For this reason it is convenient to consider the muscular and sensory supplies of the nerve trunk and of its two divisions independently.

Motor branches are given off the sciatic nerve trunk from above downwards to semitendinosus, semimembranosus, the long head of biceps, short head of biceps, and adductor magnus.

The musculo-cutaneous branch of the external popliteal nerve supplies peroneus longus and peroneus brevis: the anterior tibial branch supplies tibialis anticus, extensor longus digitorum, extensor proprius hallucis, peroneus tertius, and extensor brevis digitorum.

The muscular branches of the internal popliteal division in the order in which they arise are to gastrocnemius, popliteus, plantaris, and soleus, while the posterior tibial nerve innervates popliteus, the deep part of soleus, tibialis posticus, flexor longus digitorum, and flexor longus hallucis. The internal plantar nerve supplies abductor hallucis, flexor brevis hallucis, flexor brevis digitorum, flexor accessorius, and the first two lumbricals, and the external plantar nerve the interossei, the muscles of the little toe, the third and fourth lumbricals, and adductor obliquus et tranversus hallucis.

The action of adductor magnus and the hamstrings can best be examined when the patient lies prone with the knee flexed against resistance. The tendon of each muscle can then be palpated separately.

A suitable method of testing for voluntary contraction in gastrocnemius is to ask the patient to extend the foot while he kneels on a chair.

It should be observed whether the peronei contract synergically

with dorsiflexion of the foot in cases where this movement can be performed. The intrinsic muscles of the foot are best examined electrically.

Sensation.—The whole sensory distribution of the sciatic nerve lies below the knee. After complete division of this trunk the following sensory changes are observed. The area of loss to pressure and vibrations of a large tuning fork may comprise the whole foot with the exception of a well-defined area of somewhat variable distribution on the inner aspect of the foot and internal malleolus. This is



Fig. 14. To illustrate the average extent of loss of sensibility after complete division of the sciatic nerve trunk. The area of insensibility to light touch is enclosed by a continuous line and that to pin-prick corresponds with the cross shading, the upper borders of both areas being ill defined. The area coloured black represents the extent of loss of deep sensibility.

supplied by the internal saphenous nerve. The area of loss includes the lower part of tendo-Achillis, the external malleolus, and may extend for a varying distance on the outer aspect of the leg.

Sensibility to pin-prick is abolished over a wide area, which extends from the foot over the lower half or two-thirds of the antero-external and posterior aspects of the leg. The change from abnormal to normal sensibility is abrupt at the anterior, internal, and posterior boundaries, but the upper border cannot be so sharply mapped out (Fig. 14).

Light touch is not appreciated over the area shown in Fig. 14. The areas of loss to light contacts and to pin-prick are co-extensive, except on the upper part of the leg. Here the extent of the tactile loss exceeds that to pin-prick.

Deep sensibility need not be abolished in any part of the distribution of the external popliteal nerve. In a certain proportion of

cases pressure is not appreciated when applied to a narrow strip on the outer side of the leg and the dorsum of the foot within the areas of anaesthesia and analgesia.

When the external popliteal nerve is divided above the origin of its lateral cutaneous branch, loss of sensibility to pin-prick is found over the entire dorsal surface of the foot, and over the antero-external aspect of the leg in its lower half or two-thirds. The posterior and upper borders are ill defined in contrast to the definition of the anterior border (Fig. 15).

When the site of lesion is below the origin of the external

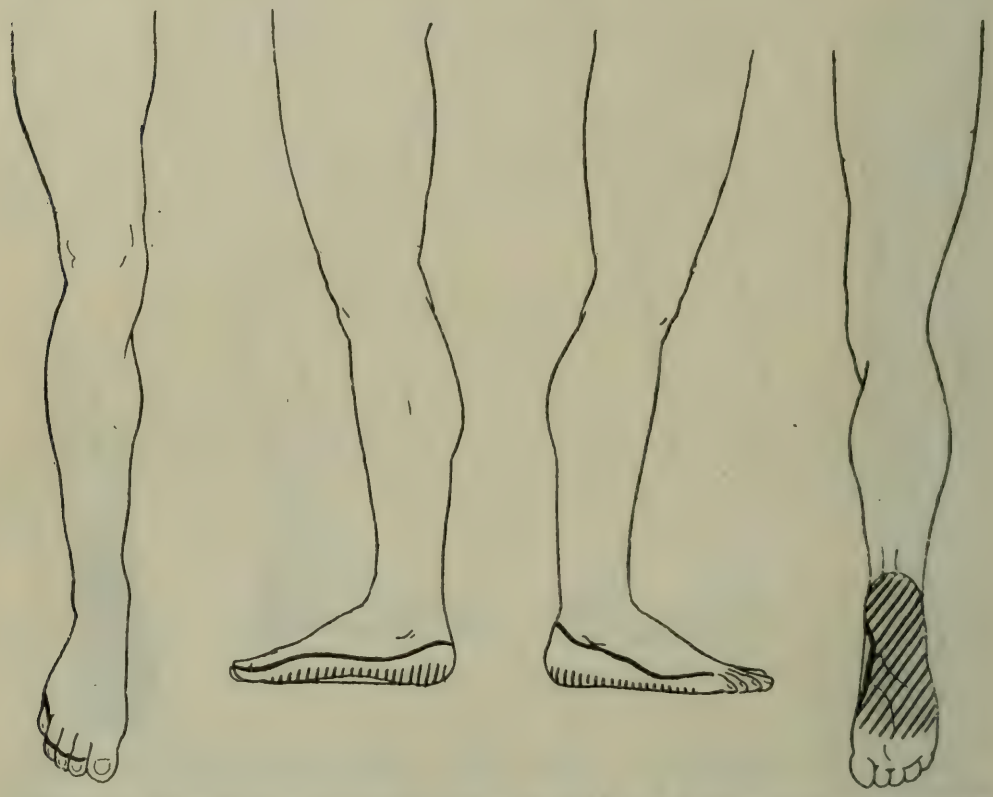


FIG. 16. Division of the internal popliteal nerve. The continuous line limits the area in which the skin was insensitive to light touch and the shaded area corresponds with the extent of cutaneous analgesia.

cutaneous branch, sensibility to pin-prick is disturbed as a rule on the dorsum of the foot only.

With complete division of the whole external popliteal nerve, the area of loss to light touch covers the dorsum of the foot, including the first phalanges of the toes, and the antero-external and posterior surfaces of the leg. This area is as extensive as that which follows division of the sciatic nerve trunk except that it does not cross the middle line in the region of the tendo-Achillis.

When the external cutaneous branch is intact, light touch is less extensively affected. The outer border of the anaesthetic area on the foot does not usually extend beyond a line running backwards from the space between the fourth and fifth toes parallel with the external border of the foot. It passes across or above the external malleolus, and does not invade the posterior surface of the leg.



Fig. 15 *a*.



Fig. 15 *b*.

Fig. 15. Division of the external popliteal nerve above the origin of the lateral cutaneous branch. To show the areas of insensibility of light touch (dotted line) and pin-prick (continuous line). From Dr. Grainger Stewart and Mr. Rowley Bristow's article in Sir Robert Jones' *Orthopaedic Surgery*.

In complete division of the internal popliteal nerve the degree of loss of deep sensibility in the sole of the foot is very variable and may be slight. The skin of the sole of the foot is insensitive to tactile and painful stimuli ; the area of loss to light touch includes the plantar aspect of the toes, while that to pin-prick does not. The average lateral limits of the anaesthetic and analgesic areas are denoted in Fig. 16.

Two wide variations from the usual clinical findings with total division of the sciatic nerve should be mentioned.

1. The upper limit of sensory supply of the external popliteal nerve has been known to reach as low as 2 in. above the external malleolus, but, before accepting any gross variation, it is important to make several examinations of the condition of sensibility.

2. A faradic response may be obtained in tibialis anticus, and its presence alone should not be taken as evidence of incomplete lesion or of recovery of the nerve.

B. *Level of Lesion.*

As stated above, the sensory distribution of the sciatic nerve lies entirely below the knee.

The external cutaneous nerve arises from the outer division of the sciatic in the popliteal space, and so may escape injury, though all the muscles supplied by the external popliteal nerve are paralysed. The presence or absence of anaesthesia on the outer aspect of the leg has, therefore, considerable diagnostic importance.

From the motor point of view the level of the lesion of the nerve trunk may be localized as being (1) above the branches to semitendinosus ; (2) below those branches, allowing the muscle to escape ; (3) below the branches to semitendinosus, semimembranosus, and the long head of biceps ; and (4) below the origin of the branches to these muscles and to the short head of biceps.

C. *Vasomotor and Trophic Changes.*

Such changes are not important as a rule with division either of the internal or of the external popliteal nerves alone. On the other hand, after complete sciatic nerve division the dry, congested, and swollen appearance of the foot when it is dependent is characteristic and prominent.

D. *The Relative Vulnerability of Internal and External Popliteal Nerves.*

Operative and clinical experience shows that the external popliteal division of the sciatic nerve suffers grosser damage as a rule than does the internal popliteal. The latter, however, does not escape injury so often as is supposed. The presence of a dropped foot not uncommonly diverts attention from the less obvious signs of an internal popliteal injury.

E. *Treatment.*

Observation stage.—The general rules for treatment by movements, massage, and electricity have already been laid down. It is very important to prevent dropping of the foot from the beginning.

For this purpose the patient must wear a night-shoe, and a splint during the day.

Operation.—When the nerve lesion is situated near the great sciatic notch, the following method of exposure is recommended.

Gluteus maximus having been exposed and its tendon divided, it is reflected towards the middle line together with the vessels and the inferior gluteal nerve. The reflection of the muscle should be carried out from below upwards. The sciatic nerve is now exposed and can be explored upwards as far as the sciatic notch and downwards into the thigh.

In dealing with the nerves in the popliteal space, great care must be taken to distinguish the internal and external popliteal nerves, for not infrequently the external division has been found displaced to an internal position. The biceps tendon has been mistaken for the external popliteal nerve.

In exposing the posterior tibial nerve in the upper two-thirds of the leg the inner attachment of soleus should be divided. The skin incision is that usually employed in the operation for tying the posterior tibial artery in its upper two-thirds.

The rules already laid down for the examination of an exposed nerve are in general applicable for the sciatic. Two additional rules for guidance in dealing with this nerve may be added.

1. The internal popliteal nerve is more important both from the motor and sensory point of view than the external popliteal. The sensory function of the external popliteal is relatively unimportant, and its motor function can be replaced by a mechanical appliance or tendon fixation of the ankle.

The principles which govern the treatment of the external popliteal nerve resemble those laid down for the musculo-spiral. The internal popliteal should be considered on the same lines as the median.

2. The size and structure of the sciatic nerve allow of partial section if this procedure is thought advisable. The internal popliteal is also large enough to be dealt with in fractions. It is even possible in some instances to suture individual nerve bundles.

In dealing with gaps in the nerve, the method to be employed in each case depends on the size of the gap.

(*a*) Relaxation by posture combined with freeing of the nerve. If the defect is not great, the ends may be brought together by extension at the hip and flexion at the knee. This posture can be maintained by a Thomas's hip splint, or by a sling round the shoulder and ankle. The patient may be nursed in the lateral or in the prone position. The hip should be released at the end of a fortnight, and the knee allowed to extend gradually in the course of the next month.

(*b*) If the gap is wide, a two-stage operation, similar to that recommended for the median nerve, can be used. It is possible to bridge a 5-in. gap with one operation, and a considerably greater defect with two or more operations.

The internal popliteal nerve is so important from the sensory point of view that all measures should be tried to bring the ends together.

Post-Operative Treatment.—This must be carried out along the same lines as during the observation stage and every effort made to prevent the development of deformities. There is one deformit

which is particularly apt to detract from the success of an otherwise satisfactory result. This is a fixed downward flexion of the terminal phalanx of the big toe, which should be dealt with by excision of the interphalangeal joint and allowing ankylosis to occur in the straight position.

F. *Signs of Returning Function.*

As a rule the internal popliteal nerve shows signs of return of function earlier than does the external popliteal, and the ultimate recovery of motor power, though not of sensibility, is better.

The following tables illustrate the length of time taken for signs of returning function to appear after suture of the sciatic nerve and of its internal and external divisions.

TABLE I

(From Professor Stopford's records.)

1. *Sciatic nerve trunk :* sensibility to pin-prick average 120 days (4 months)

	Voluntary response average.
Gastrocnemius	165 days (5½ months)
Tibialis anticus	210 ,, (7 ,,)
Extensor longus digitorum	330 ,, (11 ,,)
Extensor longus hallucis	420 ,, (14 ,,)

2. *External popliteal nerve :* sensibility to pin-prick average 90 days (3 months).

	Voluntary response average.
Tibialis anticus	150 ,, (5 ,,)
Peronei	165 ,, (5½ ,,)
Extensor longus digitorum	210 ,, (7 ,,)

TABLE II

(From the records of the Military Orthopaedic Hospital, Shepherds Bush.)

1 *Sciatic nerve trunk :*

(32 cases)	*Faradic response.*	*Voluntary response.*
Gastrocnemius	(18 cases) 398 days (13 months) (shortest 90 days, longest 1,185 days)	(8 cases) 515 days (17 months) (shortest 200 days, longest 1,185 days)
Tibialis anticus	(15 cases) 382 days (12 months) (shortest 90 days, longest 1,185 days)	(4 cases) 596 days (20 months) (shortest 200 days, longest 1,185 days)
Ext. longus digitorum	(11 cases) 367 days (12 months) (shortest 100 days, longest 1,185 days)	(4 cases) 596 days (20 months) (shortest 200 days, longest 1,185 days)
Ext. longus hallucis	(4 cases) 350 days (11½ months) (shortest 160 days, longest 515 days)	(1 case) 585 days (19½ months)
Ext. brevis digitorum	(4 cases) 350 days (11½ months) (shortest 160 days, longest 515 days)	(1 case) 585 days (19½ months)
Peroneus longus	(11 cases) 377 days (12½ months) (shortest 100 days, longest 1,185 days)	(4 cases) 576 days (19 months) (shortest 100 days, longest 1,185 days)
Tibialis posticus	(4 cases) 409 days (13½ months) (shortest 300 days, longest 515 days)	(3 cases) 373 days (12½ months) (shortest 300 days, longest 425 days)
Biceps	—	(1 case) 128 days (4 months

2. *External popliteal nerve :* (35 cases)	*Faradic response.*	*Voluntary response.*
Tibialis anticus . .	(13 cases) 319 days (10½ months) (shortest 96 days, longest 600 days)	(9 cases) 440 days (14½ months) (shortest 260 days, longest 650 days)
Ext. longus digitorum .	(9 cases) 455 days (15 months) (shortest 100 days, longest 630 days)	(6 cases) 352 days (11½ months) (shortest 270 days, longest 415 days)
Ext. longus hallucis .	(2 cases) 615 days (20½ months) (shortest 600 days, longest 630 days)	(4 cases) 415 days (14 months) (shortest 365 days, longest 500 days)
Ext. brevis digitorum .	(2 cases) 615 days (20½ months) (shortest 600 days, longest 630 days)	(4 cases) 415 days (14 months) (shortest 365 days, longest 500 days)
Peroneus longus . .	(8 cases) 309 days (10 months) (shortest 60 days, longest 630 days)	(5 cases) 405 days (13½ months (shortest 365 days, longest 500 days)
3. *Internal popliteal nerve :* (3 cases)		
Gastrocnemius . .	—	(2 cases) 200 days (6½ months)
Tibialis posticus . .	—	(1 case) 130 days (4½ months)

TABLE III

(From Colonel Sargent's records).

First evidence of return of motor function.

Level of lesion in thigh.	*Interval between wound and suture.*	*Faradic response.*	*Voluntary response.*	*Length of time after suture.*
1. Upper third	13 months	Tib. antic. & gastrocnemius	—	240 days (8 months)
2. Upper third	13 months	Tib. antic. & exten. long. digit.	—	210 ,, (7 ,,)
3. Upper third	4 months	Tib. antic. & exten. long. digit.	—	180 ,, (6 ,,)
4. Upper third	immed.	Tib. antic. & exten. long. digit.	—	180 ,, (6 ,,)
5. Upper third	—	Tib. antic.	Tib. antic.	120 ,, (4 ,,)
6. Upper third	immed.	—	Gastrocnem.	150 ,, (5 ,,)
7. Upper third	12 months	—	Gastrocnem.	120 ,, (4 ,,)
			Peronei	150 ,, (5 ,,)
8. Upper third	6 months	—	Gastrocnem.	330 ,, (11 ,,)
9. Upper third	11 months	—	Tib. antic.	90 ,, (3 ,,)
10. Lower third	immed.	Tib. ant. Peronei	—	330 ,, (11 ,,)
11. Lower third	6 months	—	Gastrocnem.	330 ,, (11 ,,)

The time taken for recovery of function to appear seems to be approximately the same whether the nerve suture is primary or secondary.

Patients in whom the functional end result is good can ascend ladders, run, and stand on tip-toe, and many of them have resumed their employment little, if at all, disabled as the result of their injuries.

9. Internal Saphenous Nerve.

Pain, sometimes causalgic in character, referred to the distribution of the internal saphenous nerve may follow its involvement in scar tissue. This pain is apt to occur in attacks which are brought on by use of the limb. It may be regarded as psychopathic in origin if an examination of sensibility is omitted. The area of tenderness is characteristic and definite, extending down the inner aspect of the leg to the ankle and foot. It is frequently associated with redness and sweating.

10. Anterior Crural Nerve.

Owing to the fatal nature of wounds in the neighbourhood of the femoral artery, cases of complete division of the whole nerve are rarely seen. Sensory disturbances resembling meralgia paraesthetica are occasionally met with and are usually due to involvement of the sensory branches, with or without the external cutaneous nerve, in dense scar tissue. For this reason they are difficult to deal with. For any quadriceps paralysis which may be present some form of tendon transplantation is advisable. Attempts to suture muscular branches are not likely to be successful.

PART III. HISTOLOGICAL EXAMINATION OF INJURED PERIPHERAL NERVES

1. Injuries of the Nerve Fibres with Intact Nerve Sheath.

Such injuries are common as a result of blows, especially in parts where the nerve lies close to a bone, for example over the musculo-spiral nerve in the middle third of the upper arm. They also occur in the neighbourhood of fractures and dislocations, as with injuries to the musculo-spiral nerve in fractures of the humerus, or to the ulnar nerve in dislocations of the elbow-joint. Similar injuries have been frequent during the late war, where a bullet or shell fragment has passed close to a nerve without actually lacerating it or tearing its sheath. The damage to the nerve fibres may assume either of the following forms in varying degree.

(i) The axis cylinders are damaged but Wallerian degeneration does not take place; they temporarily lose their normal conductivity but retain trophic power over the distal segment of the nerve. In those cases in which the injuries have been produced by the passage of a missile of high velocity exploration within a few hours has shown the nerves to be grossly swollen for a considerable distance in both directions, opaque, and presenting a yellow discoloration.

The recovery of the damaged portion of the nerve may be impeded by the pressure of effused blood or serum into or within the sheath, or by a process of fibrosis or contraction of the sheath due to neighbouring sepsis. In a simple case the function of the nerve is restored within a few days or weeks.

(ii) The damage to the axis cylinders may be so great that many or all of them are severed so that Wallerian degeneration takes place. In such a case function can be restored only by regeneration of the nerve. The conditions for this regeneration are ideal; the uninterrupted sheath prevents the escape of nerve fibrils and favours

the conduction of them toward the intact neurilemma sheaths below. At the same time the nerve is shielded from invasion by scar tissue or micro-organisms.

2. Injuries of the Nerve Fibres and of the Sheath.

In these cases the damaged nerve fibres rapidly proliferate by giving off fine fibrils, which grow out in all directions into the neighbouring tissues, and are surrounded by outgrowths from the neurolemma sheath in the form of solid protoplasmic finger-like processes. Outgrowths from the neurilemma sheath take place from the distal end of the nerve, but here the processes contain no neurifibrils. If the two outgrowths thus formed come in contact with one another, conducting neurofibrils rapidly appear within the distal end. Where no such contact is established between the upper and lower ends, the neurofibrils continue their outgrowth from the proximal end, taking lines of least resistance, such as fascial or intermuscular planes. A few may happen eventually to join the outgrowths from the distal end. The path taken by neurofibrils in passing from the upper to the lower end of the gap is always tortuous even when the ends of a nerve are brought together by suture. The question as to how far the distal end exercises any attractive influence is still unsettled.

The passage of nerve fibre processes across the gap may be impeded by either:

(1) the interposition of scar tissue; or (2) the results of infection.

(1) A considerable increase of fibrous tissue is frequently found in relation to injured nerves, even where there has been no clinical evidence of microbic invasion, but it is greatest when prolonged infection has occurred. It may be due to the organization of blood clot, necrosis from the original trauma, or the presence of foreign bodies. The nerve fibre processes appear to be unable to push aside or work their way through a scar. Individual nerve fibre processes do pass for some distance along the planes of connective tissue, especially in the neighbourhood of blood vessels, but they rapidly become scarcer and disappear as they are traced onwards. Such a diffuse outgrowth of nerve fibres must be distinguished from a 'nerve bulb' or 'amputation neuroma', which consists of twisted nerve fibre processes with a very small admixture of fibrous tissue derived from the endoneurium. This formation is the result of the normal regenerative activity of a healthy nerve.

(2) Microbic infection prevents regeneration in two ways.

(*a*) It diminishes the formation of nerve fibre processes by direct toxic action on the nerve fibres.

(*b*) It leads to fibrosis, both in the surrounding tissues and in the interstitial tissues of the nerve. Infection may ascend in the nerve for very considerable distances. The work of Orr and Rows suggests that in many cases it travels as far as the dorsal root ganglion and even the spinal cord. We have had no opportunity of verifying this observation in the wounds of the late war, but a nerve was examined by one of us (J.S.B.S.) in which there was definite evidence of ascending neuritis 8 inches above the level of the lesion. This

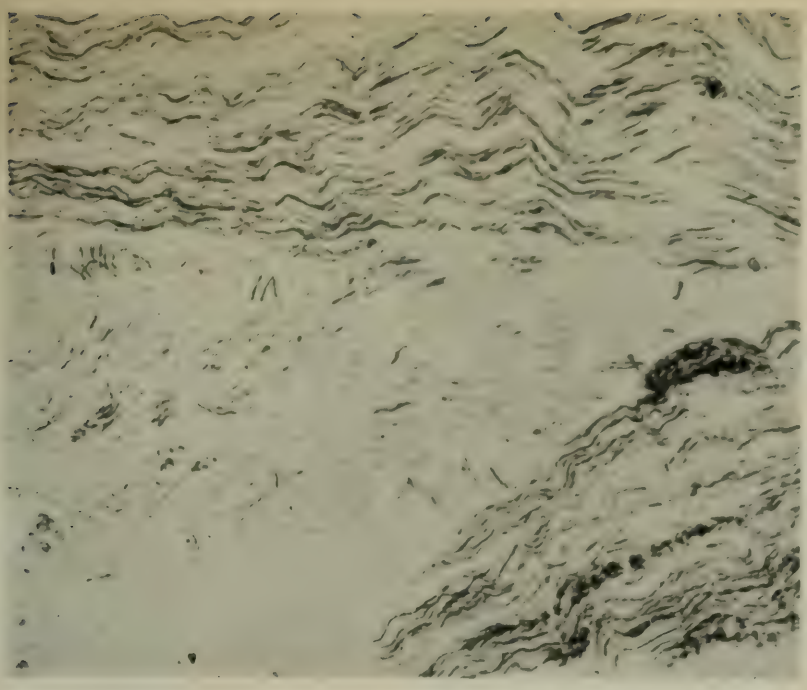


Fig. 17 *a*. Ulnar nerve 148 days after the injury. Longitudinal section through the upper end of the neuroma. Most of the new nerve fibres are still running in the old sheaths, but a few are breaking away into the neighbouring fibrous tissue. Magnification about 150 diam. Bielschowsky's method.

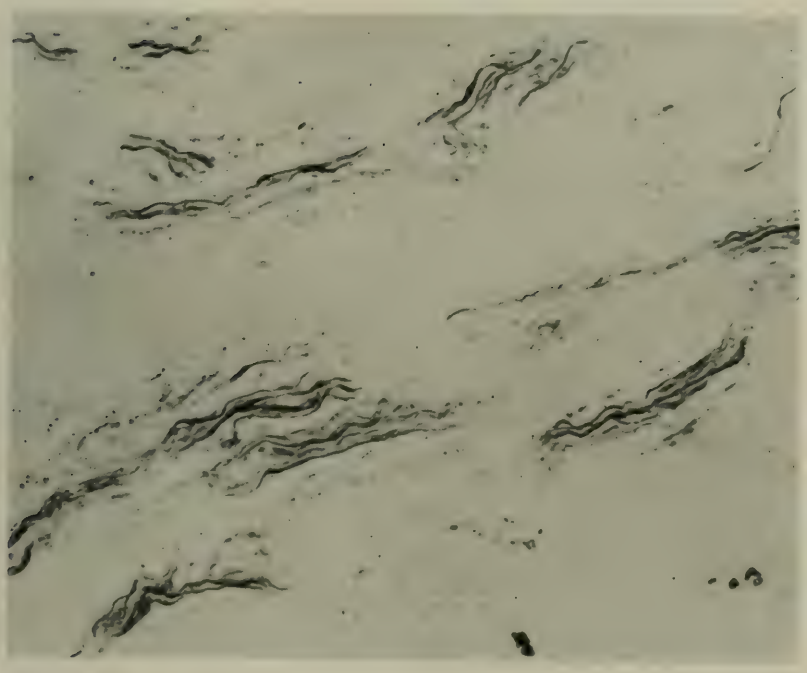


Fig. 17 *b*. Ulnar nerve 148 days after the injury. Longitudinal section through the middle of the neuroma. The new fibres are running in sparse bundles among fibrous tissue. Magnification about 250 diam. Bielschowsky's method.

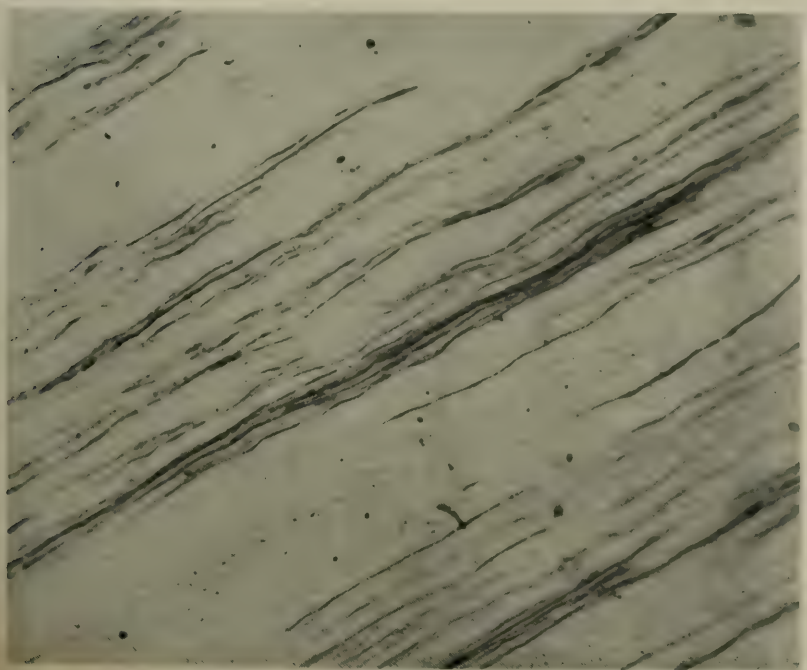


Fig. 17 *c*. Ulnar nerve 148 days after the injury. Longitudinal section through the peripheral end. New nerve fibres running in old channels of the neurolemma. Magnification about 200 diam. Bielschowsky's method.

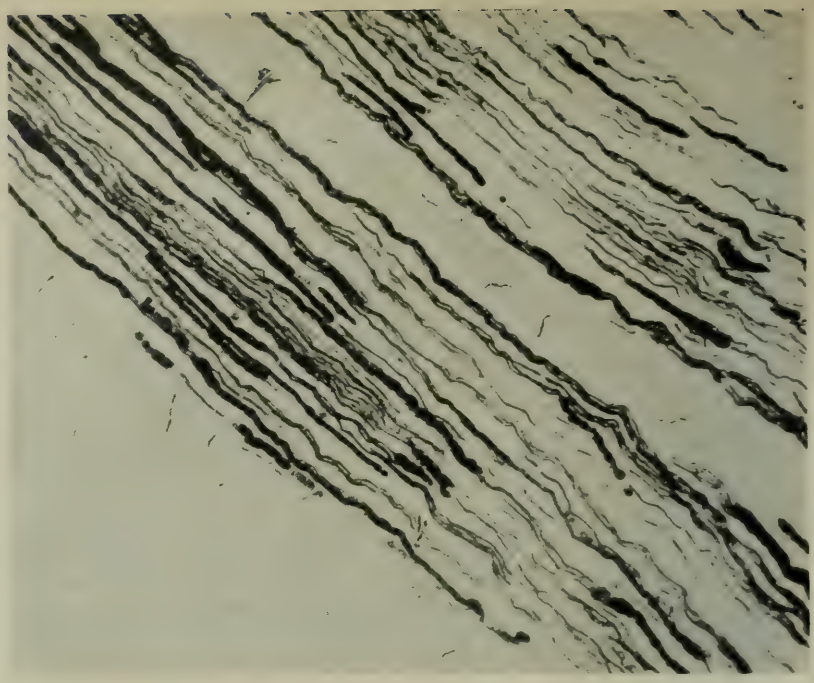


Fig. 18 *a*. Ulnar nerve 240 days after the injury. Section through the proximal end showing the beginning of the neuroma. A few new nerve fibres are leaving the old sheaths and penetrating into the neighbouring fibrous tissue. One or two of the old thick myelin sheaths are seen. Magnification about 100 diam. Wergent-Pal method.

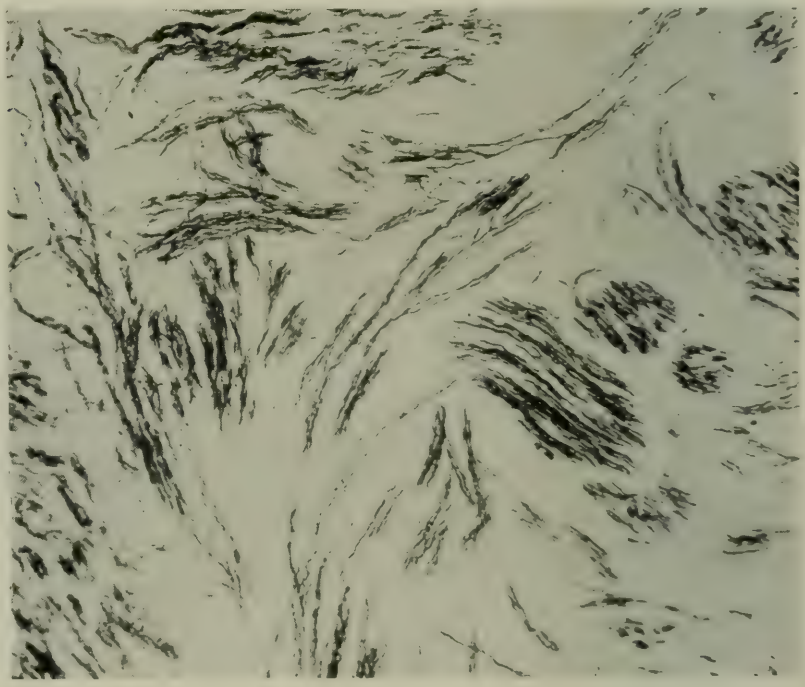


Fig. 18 *b*. Ulnar nerve 240 days after the injury. Longitudinal section through the neuroma. Here the nerve fibres have broken up into a flexiform arrangement in an attempt to regain the nerve sheaths beyond the injured portion. Magnification about 100 diam. Wergent-Pal method.



Fig. 18 *c*. Ulnar nerve 240 days after the injury. Longitudinal section of the distal end. A few nerve fibres have regained the old nerve sheaths. The width of the nerve bundle is shown by drops of degenerated myelin (black). Magnification about 100 diam. Wergent-Pal method.

interstitial neuritis is shown by a general thickening of the fibrous tissue throughout the substance of the nerve. The nerve bundles and the individual nerve fibres are separated from one another by connective tissue which is in many places oedematous. The myelin sheaths are thinner than normal, and in longitudinal sections it is often seen that the point from which nerve fibres arise is put back to a distance which may amount to an inch or more from the level of the lesion. As the nerve is traced upwards the fibrous tissue becomes more limited to the epi- and perineurium, and the last trace of it is usually a thickening of the sheath. If the sheath of the nerve at the level of section can readily be pulled back from the nerve fibres for one or two millimetres it may be assumed that a part of the nerve has been reached sufficiently free from interstitial neuritis and therefore suitable for suture.

General Results of Examination of Completely and Incompletely Divided Nerves.

(1) When a nerve has been incompletely divided, the proximal and distal cut surfaces necessarily remain fairly close together and regeneration may take place satisfactorily unless interfered with by infection. Where delay in regeneration occurred in war wounds of this kind, it was frequently found to be due to interstitial fibrosis of the proximal end of the cut portion of the nerve. Usually, especially in the smaller nerves, there was also damage to the apparently intact portion, of which a larger or smaller proportion of the fibres had broken up into a plexus-like neuroma.

(2) When the division has been almost complete, leaving the two ends united by a few strands of the connective tissue of the sheath, end bulbs have been found on the upper and lower portions and a variable number of nerve fibres have passed along the strand of tissue between them.

(3) When the two ends have been completely separated, a few fibres have sometimes been found to have passed by devious routes to the lower nerve bulb, and thus to have joined the peripheral portion, but in no such case did sufficient fibres pass to lead to any useful return of function.

PART IV. PAIN WITH INJURIES OF PERIPHERAL NERVES

A striking feature of peripheral nerve injuries in the war has been the absence of pain in the majority of cases. Not only does the patient often say that immediately he was wounded the limb felt as if it had been shot off, but painful sensations arising spontaneously may never be complained of throughout the whole period during which he is under observation.

When pain is associated with nerve injuries it is referred to the distribution of the nerve. Several varieties may be recognized, of which it is useful for clinical purposes to distinguish three.

(1) Pain, apparently spontaneous, of which causalgia and painful phantom limbs are the most important varieties.

(2) Pain evoked by stimulating a recovering nerve area.

(3) Pain evoked only by mechanical stimulation.

1. Causalgia.

This name refers to that form of burning pain first described by Paget (6) and named by Weir Mitchell (10). It is intense and persistent in nature, and is subject to exacerbations which may be excited by various forms of stimulation. These paroxysms can be evoked not only by physical contact but also by anything which excites an emotional reaction in a highly sensitive patient. For example, a sudden noise, a bright light, the sight of a wide space or traffic in the street, the visit of a friend, or the anticipation of an examination of the limb may be sufficient. The exacerbations are associated with intense mental distress which tends to make the whole condition progressively worse.

The onset of the pain usually dates from the first week or two after the injury.

A. *Symptomatology.*

In a typical case the hand is pink and moist with sweat; the skin is thin, tight, smooth, and glossy; the nails are curved, grow rapidly, and are exquisitely tender; the joints are stiff and swollen; the finger-pads are wasted, so that the nail beds protrude, and the bones are rarefied and brittle. The extreme sensitiveness of the limb is shown by the jealous way it is cherished by the patient.

Tenderness may be superficial or deep or both combined. When superficial it may extend over the whole area of the skin which in any way derives its sensibility to pain from the damaged nerve. Thus in the case of the ulnar nerve it may extend on to the radial half of the palm, or in the case of the median nerve it may spread over the ulnar half almost as far as the hypothenar eminence. All forms of stimulation accentuate the pain. In a small group of cases the tenderness is limited to deep-seated parts and is evoked only by deep stimulation. The physical changes described above are usually absent in these cases.

It should be remembered that causalgia may be accompanied by little or no paralysis within the distribution of the affected nerve.

B. *Nerves with Injury of which Causalgia is most frequently associated.*

The table of occurrence made up from Professor Stopford's records shows the preponderating association of causalgia with lesions of the median and sciatic nerves.

TABLE IV.

	No.	*Level of lesion.*
Median	56	(arm 33, forearm 19, wrist 4)
Musculo-spiral	2	
Ulnar	2+	
Brachial plexus	4	
Sciatic	32+	
Internal popliteal	6+	
External popliteal	1+	
Posterior tibial	4	
Cauda equina	1	

C. *Macroscopical Findings.*

As a rule, comparatively slight damage to the nerve is found at operation. The findings in Professor Stopford's cases are briefly given below.

TABLE V.

	No. of cases.	*Pathological changes.*
Sciatic	23	15 lacerated, 6 adhesions.
Internal popliteal	3	2 lacerated, 1 adhesion.
External popliteal	2	Both lacerated.
Median	39	31 lacerated, 8 adhesions.

D. *Diagnosis.*

The radiating, disagreeable pain evoked by stimulation of an area of recovering sensibility must be clearly distinguished from that of causalgia. Causalgic pain has the following characters. It is (1) spontaneous ; (2) hot and burning in character, intense, diffuse, persistent, but subject to exacerbations. (3) Causalgic pain can be excited by stimulation which does not necessarily produce a physical effect on the limb. Emotional excitement usually aggravates the pain and brings on a paroxysm. (4) It tends to lead to profound changes in the mental state of the patient.

On the other hand, the part to which sensibility is returning as the result of regeneration is not usually the seat of spontaneous pain. The sensation evoked by pricking the skin within such an area tends also to radiate, but it is usually described as ' tingling ', or ' pins and needles ', and has not a burning quality. It does not extend beyond the area of sensory loss due to the lesion of the nerve.

E. *Treatment.*

Before determining the lines of treatment to be followed, the physician must form an estimate of the patient's psychical state. The nature of the malady and the persistent character of the pain should be carefully explained to him as well as the broad methods of treatment to be adopted. The injury of the nerve is incomplete, and there may be but slight loss of muscular power and sensibility, so that measures involving further and prolonged loss of function should not be undertaken lightly. At the same time, unless the patient can tolerate every day free passive movement of the part, degenerative changes in the joints, bones, and soft tissues are likely to develop, particularly where the hand and fingers are involved, and these may result in grave disabilities. The pain in severe cases may last for a year and is likely to be incessant. There are local applications and drugs capable of relieving the pain to some extent for a time, but the patient must co-operate with the physician in trying to reserve drugs, as far as possible, for night use only.

By dividing the nerve completely, excising the damaged portion and re-suturing the nerve, the pain in all probability will be completely abolished, in an early case ; but the functional recovery of the nerve will take many months and may never be perfect.

If the patient is of a stable type, bears the pain well, and is getting sufficient sleep with little or no help from drugs, operative measures

will, as a rule, be unnecessary. When, however, he is unduly sensitive or obviously neurotic, and frankly expresses a dread of facing a long period of pain, radical treatment should be adopted early, either injection of the nerve with alcohol above the site of the lesion or division with suture.

But when the causalgia is not severe and interference with the function of the nerve is slight, psycho-therapeutic treatment will be found to be of great value and may allow the nerve to be conserved while preventing the development of a serious neurosis.

Local applications.—1. Moisture. In some cases heat affords relief, in others cold. Thus many patients keep a sponge beside them and are continually moistening the hand with water at the temperature which, from experience, they find to be most comforting.

2. *Evaporating lotions.*

(*a*) Linimentum Betulae Co. (B.P.C.)

(*b*) Camphor
Menthol
Chloral āā q.s.

Drugs.—1. Aspirin
Pyramidon āā gr. v. Ft. pulv.

2. Aspirin
Pyramidon āā gr. v
Heroin hydrochlor. gr. $\frac{1}{12}$. Ft. pulv.

3. Aspirin
Pyramidon āā gr. v.
Heroin gr. $\frac{1}{12}$
Veronal gr. iii. Ft. pulv.

4. Heroin hydrochlor. gr. $\frac{1}{6}$
Tabloid placed under the tongue.

5. Heroin hydrochlor. gr. $\frac{1}{6}$
Hypodermically in emergencies.

Drugs should be reserved for the night if possible. If given freely they aid in breaking down the patient's resistance to pain and thus aggravate the whole condition.

Little benefit can be hoped for from the use of electricity.

Immobilization of the limb is bad treatment. The patient must be allowed to vary its posture as he likes in his efforts to get some relief from his pain. This is of importance and helps considerably. The patient craves for variety in any form, provided he can determine it himself.

Operative treatment.—If operation is decided upon, the nerve must be dealt with radically. Care must be taken that the ulnar is not involved in the scar, even where the causalgia appears to be of median origin only, and the internal saphenous where the sciatic is at fault. These nerves are frequently injured in pairs and causalgia may be associated with all.

Mere freeing of the nerve in our experience has been valueless.

There are two operations, either of which may give beneficial results:

1. Resection of a portion of the damaged nerve followed by suture.

2. Injection of the nerve with alcohol well above the site of injury.

A 70 per cent. solution has been found to give as good results for relief of the pain as absolute alcohol, and probably does not produce the same extent of degeneration in the nerve. It is important that the needle should be inserted once only, to minimize leakage. Though there is reason to think that distension of the nerve is not of great importance, it may be best to inject a sufficient quantity of the fluid to balloon out the nerve and to give an ivory whiteness in the swollen area.

2. The Nervous Sequelae of Amputation.

Normally after all amputations phantom limbs are present for a shorter or a longer time, but the stump is painless.

A. *Painful Phantom Limbs.*

Symptomatology.—The normal phantom is one of which the patient admits the existence on questioning, and which may be associated at an early stage with occasional pain. Its tendency is to become less obtrusive and less painful and to disappear after a variable lapse of time. But in some cases pain or discomfort becomes a dominant feature. The pain or discomfort may be described as ' a cramp ' or ' drawing feeling ' associated with a fixed or varying position of the part which appears to be the site of the abnormal sensation. The patient may say, ' my fingers are bent and the nails are being driven into the palm of the hand ', or ' my knuckles are being crushed as if being held in a vice '. In other cases the sensation complained of is referred more to the surface, and the patient says that ' the skin is hot and burning ', and ' the hand seems swollen ', and ' as if it would burst '. Both deep and superficial forms of sensation may appear to be present in the same hand.

After the amputation in the majority of cases the phantom limb, painful or otherwise, is not more than a transient episode. In a small number of cases, however, the persistence and the painfulness of the phantom constitute a serious complication. Of this minority a number tend to ultimate recovery within a period of months or years, but some have been known to last indefinitely and render the patient's life intolerable.

Painful phantoms have been more common in consequence of the wounds of war than of civil practice. The probable explanation of this fact is the prevalence of infection.

Treatment.—After amputation all nerves, when left free in the tissues, form bulbs and there is no evidence that this in itself is a pathological phenomenon. The great majority of these nerve bulbs do not give rise to any troublesome symptoms. But when there is a painful phantom it is commonly found that the nerve bulbs are unusually tender. It has been a common practice to remove these bulbs, and this procedure is sometimes followed by relief of the pain. Success is more likely to follow a free removal of the nerve higher up. Many patients are much relieved by this procedure. Division of posterior roots has been frequently resorted to, and although this is not often attended by immediate relief,

experience has shown that the ultimate outlook is improved by this operation. It must be remembered, however, that the operation is a severe one.

In the treatm ent of conditions where pain is the principal symptom there are certain important factors common to all cases. Resistance to pain varies greatly in different individuals and tends to become progressively lowered. Early treatment is therefore imperative. Any surgical procedure, however, should be considered in the light of the personality of the patient, and must be associated with measures directed towards the preservation of his psychical equilibrium. For this reason sedative drugs should be avoided as much as possible and the patient should be encouraged voluntarily to give up their use. The course of his disease and the treatment adopted should be explained to him and his co-operation encouraged in every way. He must be told that the physician cannot accept the whole responsibility for success or failure ; he should not be permitted to relax his efforts and to fall into the habits of a chronic invalid. He should be encouraged daily to occupy his mind with some definite work, if possible associated with the more vivid interests of his life. External worry must be removed and deep-seated anxieties carefully investigated. In all patients with chronic pain sleep is troubled and nightmares are of common occurrence. These morbid symptoms require careful consideration, and their underlying causes must be removed if possible.

B. *Tender Stumps.*

A satisfactory stump is one which is sufficiently painless to bear an artificial limb within three months.

The chief causes of pain in the stump may be defined as : (1) sinus, (2) disease of the bone, (3) nerve bulb exposed to pressure, (4) fixation of nerves by scar tissue, (5) inflammation of tissues other than the nerves, (6) foreign bodies.

Diagnosis.—Radiographic examination is essential. A sinus may be overlooked in a pucker in an apparently soundly healed scar. If the X-ray photograph shows nothing abnormal and there is no sinus in the scar, the cause is most likely to be found in the condition of the nerve. Under such circumstances the trunk of the nerve will be unusually tender. It is not necessarily the main nerve trunk that is caught in the scar. It is therefore necessary to localize with the greatest precision the tender point or points. The possibility of a spur of bone, tender to pressure, being present should not be overlooked.

Treatment.—The majority of painful bulbs can be dealt with successfully by dividing the nerve as high above the bulb as possible, crushing, transfixing, and ligaturing the end, and injecting the nerve with absolute alcohol. The bearing points of the artificial limb to be used must be considered in deciding the point of division of the nerve. Some stumps are more difficult to deal with than others. For example, a short arm stump near the brachial plexus is particularly liable to be painful. In some of these cases pain persists in spite of division of the nerves as high as possible. It is known that

inflammatory changes can be traced centrally for a considerable distance within the nerve, and this may account for the lack of success in relieving pain by high division. On the other hand, there is much evidence to show that long-continued pain may persist after removal of every possible peripheral cause as, for example, after division of all the posterior roots innervating the amputated limb. This strongly suggests that long-continued pain can set up persistent disturbances of function in the central nervous system.

The possibility of high infection is an indication for dividing the nerve at a higher level in the limb, if excision of the bulb has failed to give relief after the lapse of three months. If even this high division proves unsuccessful after the same length of time, the question of dividing posterior roots arises.

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ADDENDUM.

Since the proof of this report was finally passed for press the Committee have had further opportunities of examining patients on whom nerve-grafting had been employed. This investigation has shown that in exceptional cases grafts have produced more successful results than previous experience had justified any hope for.

In the case of an *ulnar nerve*, divided in the upper arm, a gap of $3\frac{1}{2}$ inches had been filled by a portion of the internal cutaneous nerve removed from the same limb.

At the end of thirty-nine months flexor carpi ulnaris and flexor profundus were acting forcibly, voluntary contraction could be obtained in abductor minimi digiti and in adductor pollicis, and a faradic response in the fourth dorsal interosseous in addition to the above-mentioned muscles. Sensibility to pin-prick on the little finger was very imperfect, and appreciation of cotton wool was

completely wanting. In this case the median nerve had also been divided, and at the same operation a gap of similar length had been bridged by a piece of sciatic nerve taken from a freshly-amputated limb. Recovery had occurred to a much less extent than in the ulnar nerve. Voluntary contraction and faradic excitability were present in the deep and superficial flexors of the fingers, with the exception of those of the index and those of the thumb. There was no sign of recovery in the intrinsic muscles of the hand supplied by the median. There was no evidence of sensory recovery within the median area, except for a doubtful response to deep stimulation of the terminal phalanges of the index and middle fingers.

It should be noted that the brachial artery also had been injured and ligatured. The circulation in the hand was good and no gross vasomotor or nutritional changes were present.

From the point of view of function, the recovery in the limb as a whole was excellent. In estimating this unusually good result full allowance must be made for the fact that a very exceptional amount of care had been bestowed on the limb. Great pains had been taken not only in postural and nutritional treatment but in the re-education of movement. Further, the patient himself had given the most willing and intelligent co-operation throughout.

In the case of a *musculo-spiral nerve*, a 6 cm. graft of a human median nerve, which had been preserved in alcohol, was used to bridge the gap. Six months later a faradic response was obtained in supinator longus and extensor communis digitorum. Improvement progressed steadily, and the condition twenty-eight months after the operation was as follows. Excellent voluntary power was present in supinator longus and the extensors of the wrist. Although the patient was unable to extend the fingers, he could maintain them extended when placed passively in that position. The three extensors of the thumb were still completely paralysed. A faradic response was obtained in the extensors of the wrist and fingers, and in extensor ossis metacarpi pollicis (abductor longus pollicis).

As the outcome of a grafting operation this result may be considered exceptionally good : nevertheless it compares unfavourably both in point of time and completeness with the ordinary result of an end to end suture of the musculo-spiral nerve.

The Committee has also had the opportunity of examining two cases in which a gap had been bridged by methods of ' lateral implantation '.

In the case of an *ulnar nerve* which had been divided in the middle of the forearm, an incision had been made across one-third of the median nerve, and the proximal cut end of the ulnar had been sutured to the distal face of the section. A similar incision across one-third of the median nerve had been made $3\frac{1}{2}$ inches lower down, and the distal end of the divided ulnar sutured to its proximal face. Examined two years later, the condition was as follows. Doubtful voluntary contraction was present in abductor minimi digiti, but both this muscle and abductor indicis responded to faradic stimulation. Pin-pricks were appreciated within the whole ulnar area except over the terminal phalanx and the ulnar border of the middle phalanx of the little finger. Deep sensibility was present in the whole ulnar

distribution. No defect could be demonstrated in the functions of the median nerve.

In the case of a divided *median nerve*, the proximal and distal segments had been implanted laterally into the sheath of the ulnar nerve, every effort having been made not to injure any fibres of the ulnar. Examined thirty-four months after this operation, the condition was as follows. The radial border of the metacarpal bone of the thumb could be distinctly seen and felt, but the thenar eminence as a whole was very little wasted. The abductor brevis pollicis reacted briskly to a strong faradic current, although it was doubtful whether the patient could contract the muscle voluntarily. The action of the opponens, whether voluntary or to faradic stimulation, could not be definitely decided. The patient was able to appreciate pin-pricks over almost the whole of the terminal phalanges of the index and middle fingers. Sensibility to cotton wool was wholly absent. Deep sensibility was perfect, but the lesion of the nerve was too low for it to have been originally affected.

The result of these investigations in no way alters the view, expressed in the text of the Report, that nerve-grafting should only be adopted as a substitute for end to end suture in those very rare instances where it is absolutely impossible to bring about direct approximation.

The Committee is greatly indebted to Mr. J. L. Joyce for bringing before it the cases of lateral implantation briefly described above.

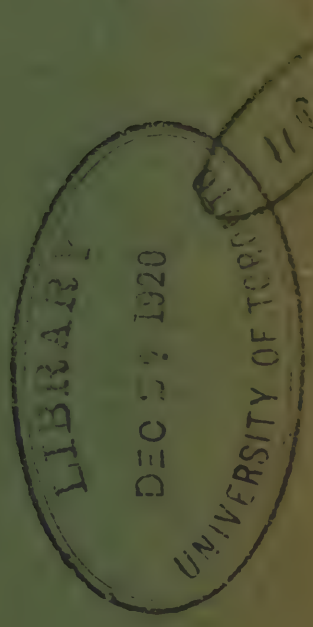
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MEDICAL RESEARCH COUNCIL

I. Results of the Examination of Tissues from Eight Cases of Death following Injections of Salvarsan.

II. The Influence of Salvarsan Treatment on the Development and Persistence of Immunity, as indicated by Measurements of Agglutinins.





LONDON
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1920

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Medical Research Council.

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Salvarsan Committee.

In February 1918 the Medical Research Committee, after consultation with the Medical Departments of the Admiralty, War Office, and Local Government Board (now the Ministry of Health), invited a Committee to consider the methods of manufacture, biological testing, and clinical administration of salvarsan and its substitutes used in this country, and the results of these, and to propose specific investigations aimed at improving those methods and results. The present constitution of the Committee is as follows:—

Sir Humphry Rolleston, K.C.B., M.D. (*Chairman*).
Professor Sir Frederick W. Andrewes, O.B.E., M.D., F.R.S.
Professor William Bulloch, M.D., F.R.S.
H. H. Dale, C.B.E., M.D., F.R.S.
Brevet-Colonel L. W. Harrison, D.S.O., K.H.P.
Major C. F. White, O.B.E., M.B., R.A.M.C.
F. J. H. Coutts, M.D., B.Sc. (*Secretary*).

INTRODUCTION

In their first Report (Special Report Series, No. 44) the Salvarsan Committee referred to some outbreaks of toxic jaundice, attended by a high fatality, which had occurred in certain military hospitals, chiefly during the year 1917. The explanation of these calamities, and especially of their occurrence in epidemic form, was, and is still, obscure. A scrutiny of the batch numbers of the samples of salvarsan, or of its substitutes, which had appeared to be responsible for the causation of the jaundice, made it quite clear that the outbreaks were not due to the use of any specially toxic batches of the drug. Two hypotheses presented themselves as offering a possible explanation of the phenomena. The first was that of infection, advocated by Professor Stuart McDonald in the *British Medical Journal* (1918, i, 76) on the ground of certain bacteriological observations made by him in cases of acute toxic jaundice following the administration of salvarsan at Newcastle. This hypothesis assumes the production by the drug of a special vulnerability of the body, and particularly of the liver, to bacterial infection, or alternatively a special vulnerability to the drug produced by infection. The second hypothesis attributes the acute liver changes to the arsenical preparation itself, and the occurrence of localized outbreaks of fatal jaundice to circumstances attending the administration of the drug, and possibly to lack of due precaution in regard to the size and spacing of the doses.

It was clear to the Committee that much information might be gained by a thorough histological examination of material from fatal cases. Dr. H. M. Turnbull, of the London Hospital, was good enough to undertake this at the request of the Committee, and he has here reported on material from eight cases. Information was wholly lacking as to any effect of arsenobenzol in lowering the resistance of the body to bacteria, such as is demanded on the hypothesis of infection. The Committee believed that evidence might be obtained by experimental work on animals, and they invited Dr. Ainley Walker, of Oxford, to undertake a series of observations on the subject. His report upon these is also given here. The Committee hope to publish a review of the whole subject, but in the meanwhile it seems to them desirable to issue the present Reports by Dr. Turnbull and by Dr. Ainley Walker in separate form.

(6535). Ps. 3912. Wt. 42443. 283/447. 10/20. O.U.P

Dr. Turnbull's material was derived from fatal cases of toxic jaundice following salvarsan at the Cherry Hinton Hospital and at certain Canadian hospitals. The material was not always adequate, and in some instances was not in the best condition for histological investigation. Nevertheless, the care with which Dr. Turnbull has performed his task and the acumen with which he has reviewed his evidence have enabled him to draw certain conclusions. It will be found that these are, on the whole, adverse to the hypothesis of an infective cause of the liver changes, and favourable to the view that the condition is a direct toxic effect of the arsenical drug.

Dr. Ainley Walker's researches have followed new lines. He decided to study the immunization response to definite doses of killed bacteria, and to ascertain the effect of salvarsan upon the agglutinin curve in rabbits when the drug was given before, during, and after the process of immunization, comparing the curves obtained with those seen in the normal animal. The evidence which he has brought forward in no way supports the view that arsenobenzol lowers the response of the body to infection. The experiments deal, it is true, with only a single kind of response—that of agglutinin production—and it would be unwise to draw wide conclusions from them as to bodily resistance as a whole. But so far as they go they do not favour the hypothesis of infection as the cause of fatal jaundice after salvarsan administration. The Committee are much indebted to Dr. Turnbull and to Dr. Ainley Walker for the careful work they have done, and would take this opportunity of expressing their thanks for these contributions towards the solution of a difficult and important problem.

MEDICAL RESEARCH COUNCIL. *June* 21, 1920.
15 Buckingham Street, Strand, W.C. 2.

I. RESULTS OF THE EXAMINATION OF TISSUES FROM EIGHT CASES OF DEATH FOLLOWING INJECTIONS OF SALVARSAN[1]

BY

HUBERT M. TURNBULL, D.M.,

Director of the Pathological Institute of the London Hospital.

CONTENTS

	PAGE		PAGE
CASE I	5	SUMMARY AND DIAGNOSIS	8
CASE II	10	,, ,, ,,	12
CASE III	12	,, ,, ,,	15
CASE IV	17	,, ,, ,,	19
CASE V	20	,, ,, ,,	22
CASE VI	23	,, ,, ,,	25
CASE VII	25	,, ,, ,,	27
CASE VIII	27	,, ,, ,,	30
GENERAL SUMMARY OF CASES, AND CONCLUSION			32
PLATES			44

CASE 1.

Private W. L. 14th K.O.Y.L.I. Aged 34. Case 11. Huts A 7 and E 6. Admitted to Cherry Hinton Military Hospital, Cambridge, June 29, 1917. Died October 24, 1917. (L.H.P.I. 582. 1917 Appendix.)

Notes received with material.

Service. Fourteen years.

History of infection. May 6 and June 6, 1917, exposed to infection. June 8, noticed discharge. June 10, noticed sore on penis.

Admitted, June 29, 1917, to Cherry Hinton Military Hospital from Ipswich, suffering from gonorrhoea.

Findings on admission: Sloughing ulcer, with hard indurated base, on glans penis encircling meatus. Phimosis. Enlargement of inguinal and posterior glands. No other clinical signs. Wassermann reaction 'strongly positive'.

Treatment: July 1, 1917, circumcised. At weekly intervals seven intravenous injections of Kharsivan (2·8 grm.), and eight intramuscular injections of mercury (8 grm.). Irrigation for gonorrhoea.

[1] This report was received on November 10, 1918.

August 23. Wassermann reaction 'strongly positive'. Potassium iodide mixture (1 ounce; 5 gr.) thrice daily for fourteen days.

September 10. Wassermann reaction 'strongly positive'. Further injections: three intravenous injections of Kharsivan (1·2 grm.), and three intramuscular injections of mercury (3 grm.).

Further history. October 1, Wassermann reaction 'strongly positive'. Patient discharged to gonorrhoeal clinic for further treatment.

October 23. Patient very ill. Tongue dry and coated. Abdomen somewhat rigid, especially in epigastrium. Liver palpable, 2 inches below costal margin. Temperature normal. Pulse 88, feeble. Slight jaundice. No vomiting. Constipation. Transferred to First Eastern General Hospital.

Death. October 24, 1917.

Report of necropsy. Body: Well nourished and not jaundiced. Lungs: Oedema; several large and many small sub-pleural haemorrhages. Heart: Normal. Abdominal cavity: Much clear bile-stained fluid. Liver: Of normal size, flabby, red, with smooth surface. Many small haemorrhages (petechial) on surface and on section, apparently round lobules. No cirrhosis. Stomach and intestines: Stomach dilated and full of altered blood. No ulceration, nor site of origin of bleeding. Duodenum and two feet of small intestine full of altered blood. Kidneys: Cloudy swelling; nothing else. Spleen, pancreas, and suprarenal bodies: Nothing abnormal observed. Head: Not examined.

Batch numbers of '606': 523 m., 524, 527, 560, 573. Kharsivan.

Macroscopic Examination.

Eight portions of liver in 10 per cent. formaldehyde solution, received from Dr. Salisbury Trevor, March 6, 1918.

The largest portion measured $2{\cdot}6 \times 1{\cdot}5 \times 0{\cdot}6$ cm., the smallest $1{\cdot}1 \times 1 \times 0{\cdot}5$ cm. In all portions there was a zone, 0·5 cm. wide, of lighter colour beneath the capsular surfaces, evidently representing the depth to which the liver had been fixed by its immersion in bulk in fixing solution.

The capsule was thin and delicately wrinkled. The cut surfaces were flat, and pale yellowish brown. On close inspection they showed a network of narrow grey lines enclosing minute, polygonal, yellowish-brown areas. In the grey lines small portal systems could be detected. The brown areas evidently corresponded to very small lobules. A few larger portal systems were present; in these the vessels and ducts were patent.

Microscopic Examination.

Two segments were cut upon the freezing microtome, and the sections were stained in Sudan and haematoxylin; four segments were put through Levaditi's process, together with a portion of liver known to contain spirochaetes; three portions were embedded in paraffin, and sections were stained by the following methods:

Ehrlich's haematoxylin and eosin; Weigert's iron-haematoxylin and van Gieson; lithium carmine and Weigert's fuchselin; Weigert-Gram and neutral red; Perl's method, reinforced by heat, for the demonstration of free iron; Gmelin's reaction for the demonstration of bile-pigment.

Liver. (Plate I; figs. 1 and 2. Plate II; fig. 1. Plate III; fig. 1). In all segments there is extensive destruction of the parenchyma. In the degree of destruction there is a slight variation in different segments and in different portions of individual segments.

In the inner third of the lobules there are no columns of hepatic cells. Within the interstitial spaces which should be filled by hepatic columns are a few, dissociated, rounded cells which are as a rule smaller than normal hepatic cells. Their nuclei are pyknotic, or faintly stained, or invisible; their cytoplasm is occasionally conspicuously eosinophil, is sometimes finely or coarsely vesicular, but is usually crowded with granules of a yellow pigment. These granules do not give the reaction of free iron, nor do they give Gmelin's reaction for bile-pigment. They are stained brown-yellow by Sudan, and appear, therefore, to be lipochrome granules (Plate III; fig. 1). In a few cells there are globules of fat. These cells remaining in the inner third of the lobules appear to be dissociated, atrophied, degenerate, hepatic cells.

In the outer two-thirds of the lobules the hepatic cells are much more numerous, and are usually grouped in continuous columns, or interrupted columns; the individual cells in the groups can seldom be differentiated. Many nuclei are absent; those that are present are structureless, and either pale or pyknotic. The cytoplasm is frequently hyaline and eosinophil; it is almost invariably rendered reticular by vacuoles. The vacuoles vary in size, but the majority are large; in sections stained with Sudan they are seen to be occupied by globules of fat (Plate III; fig. 1). There are also occasional hepatic cells which are sharply defined, smaller, rounded, and less degenerate; in one such cell a karyokinetic figure was observed.

In a zone immediately round the portal systems a greater or less number of the hepatic cells have entirely disappeared. Here many of the interstitial spaces left empty by the destruction of the hepatic columns are occupied by club-like groups of polygonal, rarely cubical, cells. These cells are smaller than normal hepatic cells; their nuclei and cytoplasm contrast sharply with the adjacent hepatic cells because they are free from degeneration. These cellular groups are usually solid, occasionally a narrow lumen is enclosed. Where such a lumen is present, it frequently contains a few neutrophil leucocytes. The free, bulbous, extremities of the club-like, cellular groups frequently lie against the peripheral extremity of a degenerate hepatic column; traced in the other direction they pass into the portal systems to become continuous with biliary ductules. These club-like, cellular groups are therefore the extremities of so-called 'pseudobile canaliculi' (Plate I; fig. 2).

There are no collections of bile within the hepatic lobules.

In the zone immediately round the portal systems there is great infiltration. The majority of the infiltrating cells are endothelial; a few neutrophil, and still fewer eosinophil, leucocytes are also present; spindle fibroblasts are exceptional. A considerable infiltration by endothelial cells and, less numerous, leucocytes extends throughout the remainder of the lobules. Some of these infiltrating cells, especially those in the zone immediately round the portal systems contain granules of fat.

There is a slight thickening of the collagen fibrils round the hepatic veins in the centres of the lobules; this thickening is not accompanied by fibroblastic proliferation (Plate I; fig. 1).

The portal systems are greatly infiltrated with endothelial cells, lymphocytes, neutrophil leucocytes and occasional eosinophil leucocytes. In the larger systems the elastic fibres are separated by the infiltration, and the intima of the portal vein is occasionally thickened by similar infiltration. In many small portal systems the elastic is destroyed to a great extent. The lumina of the bile-ducts are wide, and contain mucus and neutrophil leucocytes (Plate II; fig. 1). The lumina of the bile-ductules are small and usually empty; occasionally a single leucocyte lies in the lumen. The epithelium of many ductules contains small granules of yellow pigment which is stained by Sudan (lipochrome).

The nuclei of the infiltrating cells are pyknotic; the interstitial fibres throughout the section are swollen and haematoxylinophil; red corpuscles are not recognizable. The uniformity of these changes makes it evident that the tissue has undergone post-mortem changes before fixation.

No spirochaetes were found in the portions of tissue which were treated by Levaditi's method; spirochaetes were demonstrated clearly in the portion of congenital, syphilitic liver employed as control.

No bacteria were demonstrated either by the silver impregnation, the Weigert-Gram stain, or neutral red.

Summary and Diagnosis.

The difficulty of interpreting the finer histological changes is increased by the presence of post-mortem degeneration.

It is obvious, however, that there have been before death extensive *degeneration and necrosis of hepatic parenchyma*, the severity of which shows little variation in different lobules. The degeneration and necrosis are greatest in the central third of each lobule. Here many cells have disappeared and the remaining cells are small, and are infiltrated with lipochrome granules; infiltration with fatty droplets is rare. In the outer two-thirds of the lobules relatively few cells have disappeared, except in a zone which immediately surrounds the portal systems. In contrast with the degeneration in the central third, fatty degeneration is, here, considerable. The epithelial cells lining many of the biliary ductules in the portal systems contain lipochrome granules.

The degeneration and necrosis are accompanied by *cellular infiltration*. This is great and conspicuous within the portal systems, and in a zone surrounding the portal systems; it is considerable throughout the remainder of the lobules. The cells of the infiltration are chiefly endothelial cells and lymphocytes; in addition there are many neutrophil leucocytes and a few eosinophil leucocytes; fat-granule cells are rare. The infiltration implicates the intima of portal veins within the larger portal systems.

Considerable *destruction of elastic fibres* has occurred in the smaller portal systems.

The only evidence of *active reaction on the part of the fibrous interstitial tissue* is afforded by the presence, very rarely, of a few spindle fibroblasts in the periphery of the portal systems, and possibly also by the thickening of the collagen fibrils around hepatic veins.

Regeneration is shown by the formation of 'pseudobile canaliculi' which project from the portal systems as club-like groups of cells into the periphery of the lobules, and by the presence in the outer two-thirds of the lobules of a few, small, rounded, hepatic cells which show little degeneration; in one such cell a karyokinetic figure was observed. It is impossible to determine whether the small, rounded, hepatic cells in the central third of the lobules are regenerated cells which have undergone secondary degeneration, or are atrophied, degenerate remnants of the original hepatic cells: they certainly have undergone degeneration. The regeneration of hepatic parenchyma has not disturbed the lobular pattern of the liver.

The above changes in the hepatic tissue proper are accompanied by a *cholangitis*. This is shown by the presence of mucus and neutrophil leucocytes within the lumina of the biliary ducts, and by occasional leucocytes within the biliary ductules.

In regard to the method in which *jaundice* was caused, it can only be said that the definite cholangitis may indicate the cause, but, on the other hand, the sections show no obstructive accumulation of bile within the ductules, or intercellular canaliculi.

Estimation of the period which has elapsed since the commencement of the hepatic lesion. The determination of the duration of the lesion is obviously important. Such a determination is a matter of great difficulty, and little trust can be placed in conclusions arrived at from the examination of a single case, particularly a case in which post-mortem autolysis is present. Inasmuch, however, as this is the first case of a series it is convenient to state here the observations which my experience has led me to consider in attempting such determinations; if I have interpreted these observations correctly, attention to them in all the cases in the series should result in reliable conclusions.

Destruction of hepatic parenchyma is not invariably accompanied by reaction on the part of the fixed tissues. Almost all the parenchyma of the lobules may be destroyed and replaced by regeneration without any increase in fibrous tissue. In such cases cellular infiltration is not appreciable, and few necrosed cells are

seen ; the destructive process appears to be a relatively slight and gradual one in which individual cells are destroyed and are replaced by fresh cells. The only specimen of this kind in my possession which gives accurate information regarding time, is a liver from a patient who died on the eighth day after the inhalation of arsenuretted hydrogen ; in this specimen a large number of young hepatic cells are present in the lobules. When destruction is accompanied by the proliferation of interstitial tissue, the destruction appears to be greater in extent and intensity and more rapid ; cellular infiltration is also present. In such cases estimation of the stage of the process is easier. The infiltration precedes fibroblastic activity, and alters in character in course of time. The character of the fibrous granulation tissue gives information regarding the stage of the process, and in the later stages the appearance and development of elastic fibres form an additional criterion.

According to these criteria the liver, in the present case, has certainly undergone a severe extensive destruction. According to the character of the infiltration and the scarcity of fibroblasts this severe destruction is observed in an early stage. There has, however, been time for regeneration. Club-shaped masses of young cells have grown from the terminations of the biliary ductules. Young cells have also been formed in the more central portions of the lobules, but their number appears to be relatively small ; the post-mortem autolysis has rendered an estimation of their number difficult. It may be concluded that the liver is the seat of a recent severe destruction of parenchyma, and that there is but little evidence of this recent, severe destruction having been preceded by one more gradual and less intense.

In regard to *the cause of the extensive destruction* of the hepatic parenchyma: No bacteria were found. There is nothing in the histological picture characteristic of syphilitic infection, and spirochaetes were not detected. I found neither malarial parasites nor any accumulation of melanin or iron pigment.

Case II.

Sapper A. C. W. R. E. Aged 29. Case 15. Hut A 7. Admitted to Cherry Hinton Military Hospital, Cambridge, September 6, 1917. Died December 2, 1917. (L.H.P.I. 583. 1917 Appendix.)

Notes received with material.

Service. Thirteen years.

History of infection. None.

Admitted, September 6, 1917, to Cherry Hinton Military Hospital from Chatham.

Findings on admission: Penis normal. Marked periostitis of left tibia. No other signs of active syphilis.

Treatment: September 11 to October 24, first War-office course. The first thirteen injections were of Kharsivan, the fourteenth and fifteenth were of Arsenobillon.

October 24, Wassermann reaction 'strongly positive' at termination of course.

Potassium iodide mixture (5 gr.) thrice daily for fourteen days. Wassermann reaction 'positive'.

November 16, Kharsivan (0·3 grm.). November 17, mercury (1 grm.). November 23, Kharsivan (0·4 grm.). November 24, mercury (1 grm.).

Further history. November 30, 1917, reported sick. Not feeling fit. Had been working hard, gardening, up to this day. Conjunctivae found to be slightly jaundiced. No other clinical signs. Transferred to First Eastern General Hospital. Venesected (15 oz.) and transfused with saline. Haematemesis. Very drowsy.

Death. December 2, 1917.

Report on necropsy. Body: Well nourished, slightly jaundiced. Lungs: Nothing abnormal. Heart: A few haemorrhages along course of coronary vessels. Abdominal cavity: Some free fluid. A few haemorrhages on peritoneal surfaces. Liver: Normal size. Petechial haemorrhages on outer surface. Outer surface smooth. No abnormality observed on cut surface. Stomach and intestines: Stomach full of altered blood, from capillary oozing along greater curvature. Duodenum and jejunum full of altered blood. Kidneys: Large; some cloudy swelling.

Batch numbers of '606': 560 and 573, Kharsivan; 245, Arsenobillon.

Macroscopic Examination.

Ten portions of liver in 10 per cent. formaldehyde solution received from Dr. Salisbury Trevor, March 6, 1918.

Six portions measured each about $3 \times 1 \times 0{\cdot}5$ cm., and were without capsule; the remainder measured about $2 \times 1 \times 0{\cdot}7$ cm. and included capsule. The capsule was thin and finely wrinkled. The cut surfaces were flat. In parts they showed a grey reticulum enclosing small, polygonal, dark brown-red areas; in part they were rusty brown and almost patternless, a grey reticulum being just recognizable. The rusty brown areas were absent from the subcapsular portions, and probably represented areas of delayed fixation.

Microscopic Examination.

Nine segments were taken, and were treated in the same manner as in Case I.

Liver. Post-mortem changes are very great. Loss of nuclei is almost universal, and affects the fibroblasts, muscle-fibres, and the epithelium of the bile-ducts as well as the hepatic parenchyma; such nuclei as remain, for instance those of infiltrating cells, are pyknotic. The hepatic cells are dissociated and rounded. There is much granular débris between the cells. This appears to be derived to a large extent from disintegration of red corpuscles.

It is possible to recognize ante-mortem changes of the same nature as in Case I, but of less degree. The fatty degeneration

is less; there is less evidence of loss of hepatic cells from the centres of the lobules; cellular infiltration is less; there are no club-like expansions of pseudobile canaliculi; a few neutrophil leucocytes are present in some of the biliary ducts, but wide biliary ducts filled with muco-pus are not present.

In the portal and hepatic veins are very numerous, large, Gram-positive, oval diplococci, stout bacilli with rounded ends, and bacteria of a form intermediate between these cocci and bacilli. Some veins are completely filled by these bacteria. Similar bacteria are present in hepatic arteries and some of the capillaries.

No spirochaetes were found in portions of tissue treated by the Levaditi method, though spirochaetes were demonstrated clearly in a portion of congenital, syphilitic liver employed as a control.

Summary and Diagnosis.

The material is of little value, owing to the extent of post-mortem autolysis. According to their character and distribution the bacteria owe their presence to an agonal, or a post-mortem, infiltration and to post-mortem proliferation. They are only of significance in accentuating the secondary, post-mortem changes.

The liver is the seat of degeneration and necrosis which resemble those in Case I, but appear to have been more widespread and more rapid in action. They are associated with a cholangitis of less intensity. The absence of regenerative proliferation of the extremities of the biliary ductules indicates that the destruction of parenchyma has been more rapid and that death has occurred sooner than in Case I.

The severe autolysis renders the tissue unsuitable for the identification of haemosporidia. No spirochaetes were demonstrated by Levaditi's method.

Case III.

Private J. S. Aged 21. No. 14. Canadian General Hospital. Died January 20, 1918. Necropsy January 20, 1918. (L.H.P.I. No. 584. 1917 Appendix.)

Notes received with material.

None.

Macroscopic Examination.

Received from Lt.-Col. L. W. Harrison, on March 12, 1918, a box containing six small portions of tissue, embedded in paraffin and said to have been fixed in formaldehyde.

Microscopic Examination.

The condition in which the material was received precluded tinctorial demonstration of fat in the sections.

(1) *and* (2). *Liver* (Plate IV; figs. 1 and 2). In the central two-thirds or more of the lobules, and in areas uniting the

centres of neighbouring lobules through the points farthest from adjacent portal systems, there is a conspicuous loss of parenchyma. The interstitial fibrils which normally separate the columns of hepatic cells from the capillaries, now enclose spaces in which lie red corpuscles, polymorphonuclear leucocytes, mononuclear leucocytes, large lymphocytes, endothelial cells and occasional, free hepatic cells. The hepatic cells are usually rounded; they attain the size of ordinary hepatic cells, but are for the most part smaller. Their cytoplasm is less basophil than that of the endothelial cells; it is usually homogeneous or finely vacuolated, rarely coarsely vacuolated; it usually contains granules of yellow pigment, probably lipochrome. The nucleus has a pale nucleoplasm, a delicate membrane, indistinct, delicate threads of chromatin, and small nodes. It is frequently indented and even of kidney or horse-shoe shape; two nuclei are often present. Many of the nuclei are stained feebly; hyperchromatic and pyknotic examples are less frequent. In the most central portions of the lobules the spaces contain very few cells apart from red corpuscles, and the hepatic cells are very rare. Towards the periphery the spaces contain more cells, and the hepatic cells become ever more numerous. Here, very rarely, there is a group of two or three hepatic cells of different appearance; the cells are greatly swollen; the cytoplasm is more basophil, or is hyaline and eosinophil, and is rendered foam-like by large vacuoles; the nucleus is very degenerate or absent.

In the remaining zone round each portal system the spaces for hepatic columns contain more hepatic cells. These are of three kinds (Plate IV; fig. 2): (1) The most numerous are the less degenerate, hepatic cells, described above, which are usually abnormally small and rounded. In this zone, however, they do not contain granules of pigment. Very rarely, these cells completely fill the space, are more polygonal, and thus form a column. One cell containing a karyokinetic figure was observed. (2) The greatly swollen, extremely degenerate, polygonal, foam-like cells. These are found in occasional groups. They usually fill a portion of a space, thus forming part of a hepatic column. Occasionally, between a group of these cells and the interstitial tissue bounding the space, there are a few small, generally oblong, hepatic cells of the first type. (3) Groups of cells which are conspicuous owing to the deeper stain of their cytoplasm and nuclei. The nucleus is relatively large, oval, and has a distinct structure. The cells are, as a rule, oblong and arranged in a double row; they frequently surround a small lumen. One or more neutrophil leucocytes often lie in the lumen. These groups end blindly in the direction of the centres of the lobules; traced in the opposite direction they are seen to unite with biliary ductules. These groups are the terminations of so-called 'pseudobile canaliculi'. In the cells nearest the blind extremities the nuclei are sometimes obviously degenerate and the cytoplasm coarsely vacuolated.

The infiltration by endothelial cells and leucocytes is much greater in this zone round the portal systems than in the remainder

of the lobules. The infiltration reaches its maximum in the portal systems.

Apart from a slight excess of spindle nuclei on the collagen fibrils immediately outside the portal systems, and a slighter excess upon the fibres immediately round the hepatic veins, there is no evidence of fibrosis.

The majority of the infiltrating cells are mononuclear; polymorphonuclear neutrophil leucocytes are present, however, in considerable numbers, and are in excess in the capillaries. There is a moderate number of eosinophil leucocytes.

The sections include few large bile-ducts; the lumen of one such duct contains a little mucus and a few neutrophil leucocytes.

(3) *Cortex of kidney* (Plate II; fig. 2). The veins and the capillaries are engorged. Engorgement of the glomeruli is conspicuous. Albuminous spheres are present in a few capsules of Bowman. The cells of the first convoluted tubules are swollen. The cytoplasm towards the lumen is homogeneous and sharply defined. The basal third, or half, of the cytoplasm is split into a net by conspicuous vacuoles. Many of the nuclei are degenerate or absent. In the cells of a few second convoluted tubules the cytoplasm is vacuolar, and the nuclei are occasionally pyknotic. One collecting tubule contains a hyaline cast.

(4) *Myocardium*. The great majority of the muscle-fibres have been cut transversely. In most of the transversely cut fibres there are one or more large vacuoles. In the longitudinally cut fibres cross-striation is not seen; the contractile fibrillae are separated by minute vacuoles; large vacuoles are present in places.

(5) *Spleen*. The capillary veins of the pulp and the veins are engorged. In the centres of several of the Malpighian bodies there is a group of swollen, degenerate, and necrosed cells which have proliferated from the reticulum. Throughout the Malpighian bodies there is a slight proliferation of endothelial cells. There is a greater proliferation of endothelial cells in the sheaths of the arterioles beyond the Malpighian bodies, and here there are many eosinophil leucocytes. The reticular strands of the pulp are narrow and contain very few free cells. The number of neutrophil leucocytes is not obviously excessive.

(6) *Appendix*. The lumen is filled by red corpuscles, lymphocytes, occasional leucocytes, groups of degenerate or necrosed epithelial cells, masses of bacteria and disseminated bacteria. The mucosa is interrupted in places. These interruptions lead into lenticular cavities which extend for a wide distance in the deeper mucosa, and reach the submucosa. The cavities are filled with contents similar to those in the lumen. The majority of the bacteria are Gram-negative; the most numerous are bacilli which vary greatly in form but have rounded ends, and are for the most part short and relatively broad. The Gram-positive organisms are chiefly large, stout bacilli and yeast-like cocci. In the deeper layers of the mucosa and inner part of the submucosa some capillaries appear to be filled either by a homogeneous, very haematoxylinophil substance or by droplets of very haematoxylinophil substance;

these substances do not give the reactions of fibrin (Weigert-Gram) or calcium (silver nitrate). On closer inspection the substances are seen not to fill but to line the vessels, and to correspond in position to the endothelium; moreover, degenerate endothelial cells containing haematoxylinophil droplets can be seen in places. Gram-negative and positive bacteria lie in the haematoxylinophil substance. The haematoxylinophil substance appears to be formed by a necrosis of endothelium. The submucosa is narrow and dense. The muscularis is narrow. There is no inflammatory reaction in the tissues.

Summary and Diagnoses.

The liver is the seat of very extensive destruction of parenchyma. In the periphery of the lobules there are a few small portions of the original hepatic columns; in these the cells are very degenerate, greatly swollen, and rendered foam-like by large vacuoles. Similar cells in Case I were found to contain large droplets of fat. In this zone there are also the extremities of regenerated pseudobile canaliculi. The remainder of the parenchyma is represented by hepatic cells which are usually rounded and smaller than normal hepatic cells, and are almost always separate from one another. Their cytoplasm shows little evidence of degeneration, being usually homogeneous or finely vacuolated; their nuclei also show little evidence of degeneration, and are frequently of kidney or horse-shoe shape; double nuclei are frequent; one karyokinetic nucleus was observed. The small size of the majority of these cells, their slight degeneration when compared with the vacuolated hepatic cells in the periportal zones, and the shapes of their nuclei suggested that they might not be hepatic cells, but proliferated stellate endothelial cells of Kupffer. In certain circumstances, for instance severe malarial infection of the liver, the Kupffer cells attain a remarkably large size, and are difficult to distinguish from hepatic cells. The following observations, however, indicate that the cells under discussion are hepatic cells: their cytoplasm is more eosinophil than endothelial cells which are present beside them; they are very scarce in the centres of the lobules, although inflammatory cells are numerous there; all transitions can be observed between the smaller rounded forms and larger polygonal forms which have all the appearances of hepatic cells; in places in the periphery of the lobules they are arranged in solid columns in the manner of columns of hepatic cells. It would appear, therefore, that these less degenerate cells are atypical hepatic cells, formed by regeneration; that a few of the original cells of the hepatic columns remain as greatly degenerated, foam-like, fatty cells: that of the remainder, some have been destroyed entirely, and this destruction has been greatest in the portions of the lobules furthest from the blood supply of the portal systems, whilst others have been less affected and have multiplied by regeneration, and have given rise to unusually small, rounded, hepatic cells; that such regeneration has been greatest near the portal systems. Some of these regener-

ated hepatic cells have then undergone a degeneration, though a degeneration less conspicuous than that in the remnants of the original columns; that a degeneration has followed regeneration is proved by the degeneration of many of the terminal cells of the pseudobile canaliculi.

Blood has passed from the capillaries into the interstitial spaces in which hepatic columns originally lay. The destruction of parenchyma is associated with an infiltration which is greatest in, and in the neighbourhood of, the portal systems. The infiltrating cells are chiefly mononuclear. Polymorphonuclear leucocytes are less numerous; of the polymorphonuclear leucocytes neutrophil examples are much more numerous than eosinophil. There is no evidence of fibrosis apart from a very slight proliferation of fibroblasts about the portal systems and the hepatic veins. A slight cholangitis is present.

The changes in the liver resemble, therefore, in kind those in the liver of Case I. The better preservation of the tissue allows more certain conclusions to be drawn regarding *the progress and stage of the destructive process.* According to the interpretation of the sections given above, sufficient time has elapsed since the onset of destruction for considerable regeneration to have taken place. The extent of the original destruction had been so great that very few of the original hepatic cells remain. There is great cellular infiltration, but fibroblastic proliferation is very slight and of early character. The destructive process has been sufficiently slow and gradual for a considerable proliferation of less damaged hepatic cells to take place, but time has not been sufficient for much fibroblastic proliferation, unless, possibly, the initial destructive process was of an intensity which did not immediately excite fibroblastic activity. Ultimately, some of the regenerated cells have undergone degeneration.

In the *kidney* passive congestion and parenchymatous degeneration are present. The vacuolation of the bases of the cells of the first convoluted tubules is such as is seen in paraffin sections of these tubules when the bases of the cells are loaded with fat. The kidney appears, therefore, to have been the seat of a fatty degeneration of much greater degree than results from passive congestion.

The changes in the *myocardium* also suggest fatty degeneration.

In the *spleen* there is passive congestion. In addition slight, subacute inflammatory changes are demonstrated by 'central necrosis' in some Malpighian bodies, and a slight proliferation of endothelial cells throughout the pulp.

The mucosa of the *appendix* has been torn up by haemorrhage. Numerous bacteria of different kinds are present, in the lumen and destroyed portions of the mucosa, and a few are present in some vessels, but the absence of inflammatory reaction points to the haemorrhage having been independent of bacterial action. The peculiar haematoxylinophil necrosis of the endothelium of the vessels is possibly the primary cause of the lesion, toxic injury of the vascular endothelium having led to haemorrhage.

Malarial parasites. In the liver and spleen I could detect

neither malarial parasites nor accumulations of pigment such as occur in these organs in malarial infection.

Case IV.

Sergeant C. H. Aged ? Ward E 1, No. 14 Canadian General Hospital. Died ? Necropsy, March 3, 1918. (L.H.P.I., No. 585. 1917 Appendix.)

Notes received with material.

None.

Macroscopic Examination.

Received from Lt.-Col. L. W. Harrison on March 12, 1918, a box containing nine portions of tissue, embedded in paraffin and said to have been fixed in Zenker's solution.

Microscopic Examination.

Tinctorial demonstration of fat was excluded by the condition in which the specimens were received.

(1) *and* (2) *Liver.* There is very great loss of parenchyma throughout both portions. The histological changes are essentially similar to those in Case III. Portions of degenerate and necrosed hepatic columns are more numerous; the regenerated hepatic cells are less numerous. In the central two-thirds of the lobules haemorrhage is more conspicuous. Cholangitis is not present. No bacteria were found.

(3) *Kidney.* There is considerable passive congestion. The capsules of Bowman contain albuminous, vacuolated spherules which appear to be derived by necrosis and desquamation of the cells of the epithelial lining. Coarse vacuolation of the bases of the cells of the first convoluted tubules is conspicuous. The cells of the ascending limbs of the loops of Henle show coarse vacuolation, which is usually basal, and their nuclei exhibit much karyorrhexis and pyknosis. There is catarrhal desquamation of the epithelium of the descending limbs of Henle. Pyknosis of nuclei, vacuolation of cytoplasm, necrosis, catarrhal desquamation, and accumulation of granular material in the lumen are present in many second convoluted tubules. Similar changes of much less degree are found in the collecting tubules. Many cells of the discharging tubules contain yellow granules, presumably granules of lipochrome. The majority of all kinds of tubule contains protein granules and occasional epithelial cells; several tubules contain hyaline casts. There is extensive haemorrhage into the interlobar interstitial tissue. The arteries are of ordinary size and structure. There is no inflammatory reaction.

(4) *Lung.* There is slight over-distension of the infundibula and alveoli. The capillaries are distended by red corpuscles, and there are many red corpuscles in the alveoli. Many of the alveoli also contain a few small granules of a coagulated substance which is partly stained by Weigert's reaction for fibrin. The walls of the

alveolar passages leading out of one respiratory bronchiole contain, and are lined by, a layer of neutrophil polymorphonuclear leucocytes. The bronchioles contain red corpuscles and neutrophil leucocytes.

On the pleura lie red corpuscles, and, on part of the surface, a narrow layer of granular, albuminous substance and a small mass of degenerate and necrosed endothelial cells.

Within the purulent exudation in the bronchioles are numerous Gram-positive diplococci, which have the morphological characters of pneumococci. Similar diplococci lie in small number upon the inner surface of some of the alveoli.

(5) *and* (6) *Spleen.* The capillary and larger veins are engorged; the reticular strands of the pulp also contain many red corpuscles. In many Malpighian bodies there are small, central groups of swollen, degenerate and necrosed cells derived from the reticulum. The interstitial tissue of the reticular strands is swollen, granular, and poorly stained, so that it is difficult to determine the limits of the capillary veins. Throughout the pulp the endothelial cells show proliferation and desquamation, and there is a definite excess of neutrophil leucocytes. The nuclei of many of the leucocytes and some of the endothelial cells are degenerate. One or two endothelial cells containing the nuclei of ingested cells were observed.

In a section stained by the Weigert-Gram method and counter-stained with neutral red two Gram-positive diplococci, two Gram-positive cocci and one Gram-negative diplococcus were found.

(7) *and* (8) *Pancreas.* In section (7), lying against the pancreas, for the most part in immediate contact with the glandular tissue, is a portion of spleen, probably a portion of an accessory spleen. It shows changes similar to those described in the former sections of spleen, and, in addition, has been considerably broken before fixation. Groups of splenic cells lie against the outer surface of the pancreas, and even in spaces in the interlobar septa of both sections; these splenic cells were evidently introduced with the knife at necropsy. The pancreatic lobules which lie against the spleen are small and narrow. They show an excess of interacinar interstitial tissue, and this tissue is, in places, infiltrated with lymphocytes.

In the peripancreatic and interlobar lipomatous tissue some of the fat cells are partly or completely occupied by a substance stained by eosin; other portions of this tissue are transformed into a finely granular débris. In portions of the glandular tissue the parenchyma and interstitial tissue are well stained and appear normal. In the greater part, however, of the glandular tissue the cells of the alveoli and acini are swollen; their nuclei are usually absent, and if present are pyknotic; the interstitial fibrils are swollen, haematoxylinophil, and sometimes broken into granules; in places the cells and interstitial tissue together form a granular débris.

In the pancreatic ducts the epithelium is desquamated, for the most part in ribbons; there is no evidence of proliferation of epithelium.

In the peripancreatic tissue are many large, broad, Gram-positive

bacilli, a few large round and oval Gram-positive cocci and intermediate bacillary forms; these organisms are also present, in smaller number, in the interlobar septa.

(9) *Suprarenal body.* The section does not include medulla. The capillaries are conspicuously engorged. The cells of the reticular zone are dissociated and rounded. No bacteria were detected.

Summary and Diagnoses.

In the *liver* there is a great destruction of parenchyma. The changes are essentially similar to those in the preceding three cases. Remnants of original hepatic columns are more numerous than in Case III, but less numerous than in Case I; small hepatic cells have been formed by regeneration, but their number is less than in Case III. Dissolution and replacement of the original parenchyma has progressed further than in Case I, but has not progressed as far as in Case III. Death has occurred later than in Case I and earlier than in Case III; necrosis of the liver appears to have been more rapid than in Case III, less rapid than in Case I. The cholangitis which was present in the three preceding cases, and was most conspicuous in Case I, is not shown in the sections.

The *kidney* exhibits a very severe parenchymatous degeneration. In the epithelium of the first convoluted tubules and of the ascending limbs of the loops of Henle are appearances such as are seen in paraffin sections when severe fatty degeneration has been demonstrated in frozen sections. The appearances in the epithelium of other tubules suggest fatty degeneration. There can be little doubt that fatty degeneration was severe, and was the predominant type of degeneration. Albuminous casts are present. There is no active inflammatory reaction.

In the *lung* there is an acute, purulent, pneumococcal bronchitis associated with an early broncho-pneumonia.

The *spleen* and a *splenunculus* are considerably inflamed and congested; they are of the type of 'the red septic spleen'. Evidence that the spleen was exceptionally soft at the post-mortem examination is to be found in the fragmentation of the tissue of the splenunculus on section before fixation, and in the carriage by the knife at the necropsy of splenic pulp on to the surface and into the septa of the pancreas. There is no reason to believe that the condition of the spleen is connected directly with the condition of the liver. It is doubtless due to some accessory infection, for instance the pneumococcal broncho-pneumonia; the presence of a few Gram-positive diplococci in the spleen suggests that the pneumococcal infection was the cause.

In the *pancreas* there is lipolysis and extensive digestion of the glandular tissue. It is impossible to decide to what extent this autodigestion occurred before death, in the agony, or after death. The focal nature of the digestion is in favour of its occurrence before death. Evidence of focal, chronic inflammation is presented by the lobular atrophy, interstitial increase, and lymphocytic infiltration in the periphery of the gland. There is no evidence of acute

inflammation. Bacilli of the type found associated with post-mortem putrefaction are present in the peripancreatic lipomatous tissue and the interlobular septa.

The changes in the *suprarenal body* are such as occur in post-mortem degeneration.

No evidence of *malarial infection* was found in the above organs.

Case V.

Private H. Age? No. 14 Canadian General Hospital. Died ? Necropsy, January 19, 1918. (L.H.P.I., No. 586. 1917 Appendix.)

Notes received with material.

Clinical diagnosis: Results of injections of arsenobillon and mercury.

Anatomical diagnoses: Jaundice; acute atrophy of liver; sub-acute nephritis; haemorrhages into mesentery.

Macroscopic Examination.

Received from Lt.-Col. L. W. Harrison, on March 12, 1918, the following portions of tissue, in 80 per cent. alcohol after fixation in formaldehyde.

(1) A triangular portion of liver ($2 \times 2 \times 0{\cdot}3$ cm.). The cut surfaces showed a grey net enclosing minute, brownish-yellow polygonal areas.

(2) Portion of kidney ($2 \times 1{\cdot}5 \times 0{\cdot}2$ cm.) including cortex and a fragment of medulla. On the cut surface the cortex bulged, the labyrinths appeared swollen and of a pale clay colour, the pyramids of Ferrein were narrow and grey, and the medulla was grey brown.

(3) Triangular portion of cortex of kidney ($2{\cdot}5 \times 2 \times 0{\cdot}2$ cm.).

(4) Portion of spleen ($1{\cdot}2 \times 1 \times 0{\cdot}2$ cm.).

(5) Portion of lung ($1 \times 0{\cdot}8 \times 0{\cdot}3$ cm.).

Microscopic Examination.

Portion (3) of renal cortex was transferred to 4 per cent. saline formaldehyde in the hope of demonstrating fat with Sudan III in frozen sections. The remaining portions of tissue were embedded in paraffin.

(1) *Liver.* The tissue has undergone severe post-mortem degeneration, as is shown by the loss of affinity for the majority of stains, by laking of red blood corpuscles, by much precipitation of formaldehyde granules, and by an evenly and universally distributed pyknosis and necrosis. These changes are less severe in a zone beneath the capsule.

The other changes are essentially similar to those in the previous cases. The destruction of parenchyma is severe. There are few remnants of hepatic columns in the periportal zone. Regenerated hepatic cells such as were described in Case III can be detected,

but the putrefaction of the tissues makes it impossible to estimate their relative number. Proliferation of fibroblasts and increase in interstitial fibres round the portal systems and hepatic veins is slightly greater than in previous cases, and is greatest beneath the capsule. The intima of many hepatic veins is slightly thickened by inflammatory proliferation. The epithelium of the bile-ducts is desquamated, but the desquamation has the characteristics of post-mortem desquamation: the cells are of normal shape, and are arranged in ribbons or in groups in which the cells retain the normal relation to each other. There is no evidence of cholangitis. No bacteria were detected.

(2) *Kidney.* The kidney has evidently undergone much post-mortem degeneration: the cells of the first convoluted tubules are swollen so as to occlude the lumen and, almost without exception, are devoid of nuclei; such nuclei as remain are pyknotic or, more commonly, structureless and very faintly stained. The nuclei of the cells of the other tubules, the glomeruli and interstitial tissues are pyknotic. The interstitial fibrils are swollen and poorly stained.

The following appearances are presented by the paraffin sections. In the capsules of Bowman are vacuolated spheres, evidently derived from the epithelial lining, because degenerate nuclei are present in a few. Basal vacuolation, such as has been described in previous cases, is seen in the cells of the first convoluted tubules. Large vacuoles are present in the epithelium of the ascending limbs of the loops of Henle, and in many of the cells of the second convoluted tubules. The epithelium of the collecting and discharging tubules is desquamated. Many of the descending limbs of the loops of Henle contain hyaline casts. In the cortex, especially its outer zone, are a few small patches of fibrosis enclosing small fibrotic glomeruli and atrophied tubules. In the medulla are several cysts lined by cubical epithelium and filled by coagulated albuminous substance. The structure of the arcuate and interlobular arteries is normal. Among the necrosed cells in some of the tubules in the pyramids of Ferrein are groups of hyaline, haematoxylinophil, refractile spheres; these do not give the reaction of calcium when treated with silver nitrate.

In the frozen sections stained with Sudan the amount of fatty substance present is very striking. The vacuoles seen in the paraffin sections all contain fatty substance. The basal third or outer half of the cells of the first convoluted tubules is filled by fatty globules; the cells of the ascending limbs of Henle and the degenerate cells of Bowman's capsule are almost completely filled therewith; numerous cells of the lining of the second convoluted and collecting tubules contain a large globule; there are a few droplets in the glomerular tufts. Discharging tubules are not included in the specimen.

There is no evidence of active, inflammatory reaction. No bacteria were detected.

(3) *Spleen.* The tissue has been extensively broken before fixation. Malpighian bodies are small. The blood is laked.

There is much precipitation of formaldehyde granules. The fibrils in the reticular strands of the pulp are granular and shreddy. In the capillary veins and reticular strands are many large, free endothelial cells; the majority are faintly stained and have no nucleus; of those in which nuclei are visible, several are multinuclear. The endothelial cells lining the capillary veins lie at a little distance from the walls, are rounded, and are separate from one another. There is no appreciable excess of leucocytes. No bacteria were detected.

(4) *Lung.* The pulmonary arteries and veins and the alveolar capillaries are engorged. The alveoli, with the exception of a few in the periphery of the section, and the bronchioles are filled by red corpuscles or by a coagulated albuminous substance containing red corpuscles. The red corpuscles are not laked. In the alveoli there is a considerable number of large, free, epithelial cells. These are loaded with formaldehyde granules of which there is a great deposit throughout the section; they do not contain iron-pigment (Prussian-blue reaction). There are few leucocytes in the extravasated blood. There are many Gram-positive diplococci and, less numerous, oval or round streptococci in chains composed usually of six but sometimes of as many as twelve cocci; the majority of the diplococci have the shape of typical pneumococci. The cocci lie chiefly on or close to the alveolar walls. Gram-negative cocci of similar form are also present.

Summary and Diagnoses.

The tissues, with the exception of the lung, have undergone great post-mortem autolysis. In the *liver* there has been an extensive, severe destruction of parenchyma of the same kind as had occurred in the previous cases. There are few remnants of the original hepatic columns. Regeneration is demonstrated by numerous club-like, cellular processes at the extremities of biliary ductules; the putrefactive changes prevent an estimation of the extent to which hepatic cells have been formed by regeneration in the more internal portions of the lobules. Secondary fibrosis is of greater degree than in previous cases, but is very slight. The pathological process appears to have been of slightly longer duration than in previous cases. Cholangitis is absent. In the *kidney*, in addition to a very little chronic interstitial nephritis, there is a very severe parenchymatous degeneration. The changes in the epithelium, as seen in the paraffin sections, resemble those described in the previous cases; frozen sections stained with Sudan confirm the opinion, expressed above, that the bulk of these changes was due to an exceptionally severe fatty degeneration. In the *spleen* the majority of the histological changes must be attributed to post-mortem autolysis, but the exceptional number of large, free endothelial cells in the pulp gives evidence of an inflammatory reaction. In the *lung* there is haemorrhage. This haemorrhage is associated with no appreciable inflammatory reaction, except the occurrence in the alveoli of a moderate number of large, desquamated epithe-

lial cells. The great number of cocci, however, of the morphological character of pneumococci, indicates that the lesion is not a simple haemorrhage, but an early stage of pneumonia. *Bacteria* were not found in the other tissues.

No *malarial parasites* were observed, but the post-mortem degeneration of the greater part of the material is so great as to render their detection impossible.

Case VI.

Private J. A. G. Aged ? No. 14 Canadian General Hospital. Died ? Necropsy, March 6, 1918. (L. H. P. I., No. 587. 1917 Appendix.)

Notes received with material.

Clinical diagnosis: Late results of poisoning by arsenobillon and mercury.

Anatomical diagnoses: Jaundice; acute degeneration of liver; cloudy swelling of kidneys; slight acute catarrhal gastritis; commencing myocardial degeneration; slight atheroma of aorta.

Macroscopic Examination.

Received from Lt.-Col. L. W. Harrison, on March 12, 1918, the following portions of tissue, in 80 per cent. alcohol, after fixation in Zenker's solution.

(1) Liver ($2 \times 1{\cdot}5 \times 0{\cdot}3$ cm.). The cut surfaces showed a yellow net enclosing small, dark polygonal areas.

(2) Cortex of kidney ($1{\cdot}5 \times 0{\cdot}9 \times 0{\cdot}7$ cm.).

(3) Spleen ($2 \times 1{\cdot}5 \times 0{\cdot}4$ cm.).

Microscopic Examination.

A small portion of (1), Liver, and half of (2), Kidney cortex, were placed in 4 per cent. saline formaldehyde to be cut on the freezing microtome and to be stained in Sudan. The remaining portions were embedded in paraffin, and sections, after treatment with an alcoholic solution of iodine and potassium iodide to remove precipitates, were stained by the following methods: Ehrlich's haematoxylin and eosine, Weigert's iron haematoxylin and van Gieson's mixture, lithium carmine and Weigert's fuchselin.

(1) *Liver.* The changes are essentially similar to those in the previous cases. The tissue is better preserved; the red corpuscles are stained brightly. There is very extensive and severe necrosis of the parenchyma.

In the outer two-thirds of the lobules there are hepatic columns, but these are fragmented, and their cells are in places dissociated. The cells are almost without exception devoid of nuclei; in a few cells fragments of the nucleus are still present. The cytoplasm is acidophil and vacuolated; in many cells, especially those in the peripheral zones of the lobules, it has a spongy, or frothy, appearance. In the extreme periphery of the lobules there are a few

groups of cells at the extremity of ductules. These cells are nucleated, and show little evidence of degeneration; their cytoplasm is basophil. These groups of cells have evidently been formed by regeneration. In the central third of the lobules the destruction of the parenchyma is greater. The hepatic columns are completely broken up, many cells have evidently disappeared entirely, and those that remain have for the most part been reduced to granular débris. In the lobules there are extremely few examples of isolated regenerated cells.

In the sections stained by Sudan the vacuoles in the cellular cytoplasm are seen to contain fat. In the central third there is relatively little fat; such as is present lies almost entirely within infiltrating, phagocytic cells. The fat is much more abundant in the middle and outer thirds. Here it lies for the most part within the necrosed hepatic cells, and the globules in the cytoplasm of these cells are largest and most numerous in the outer third. The small groups of regenerated cells at the extremities of biliary ductules are usually entirely free from fat, occasionally they contain a few small granules.

With the loss of hepatic cells in the centres of the lobules are associated conspicuous widening and engorgement of the capillaries. The red corpuscles, however, are not confined to the capillaries, but have also passed into the spaces which should be occupied by columns of hepatic cells; the central portions of the lobules are, therefore, haemorrhagic.

The portal systems are infiltrated with endothelial cells, lymphocytes, and neutrophil and eosinophil leucocytes. The elastic fibres are dissociated by this infiltration, and, in the case of the smaller portal systems, appear to be fragmented. A similar, though less conspicuous, infiltration extends throughout the lobules, large endothelial cells being prominent. As mentioned above, some of the cells infiltrating the lobules, particularly those in the central third, are loaded with fat; a few of those infiltrating the portal systems are also loaded with fat.

No proliferation of the fixed tissue is recognizable.

There is no pigmentation, except fine yellow granules within a very few of the cells of the centres of the lobules.

Leucocytes are present in the lumina of the biliary ductules. There are no large ducts in the sections; the lumina of such ducts as are present are narrow and empty.

No haemosporidia were found. There is no accumulation of melanin.

(2) *Kidney* (Plate III; fig. 2). The specimen does not contain medulla. The changes are essentially similar to those described in other cases, but the degeneration is severer, and the picture is not complicated by post-mortem autolysis. There is severe albuminous degeneration of the first convoluted tubules and ascending limbs of the loops of Henle; many of the cells lining these tubules are devoid of nuclei. A severe fatty degeneration is present (Plate III; fig. 2), and resembles in distribution that described in Case V; comparison of the Sudan with the paraffin sections gives additional confirmation

to the diagnosis of severe fatty degeneration which was made in cases in which material embedded in paraffin was alone available (Plate II; fig. 2). There is catarrhal desquamation of the epithelium of many second convoluted tubules. In the majority of the collecting tubules of the medullary rays are hyaline albuminous casts, granular casts, or masses of desquamated fatty epithelium. In several tubules there are red corpuscles. There is no active inflammatory reaction.

(3) *Myocardium.* There is a vacuolation of a considerable number of the muscular fibres which is possibly due to fatty degeneration.

Summary and Diagnoses.

The changes in the liver and kidney are essentially similar to those in the preceding cases. The almost complete necrosis of the *liver* appears to have occurred with a rapidity similar to that in Case I, and with greater rapidity than in Case III. Thus in the outer two-thirds of the lobules the original hepatic columns are but little broken up. Even early fibroblastic proliferation is absent, and regeneration is only shown by the formation of a few small groups of cells at the extremities of bile ductules, and by extremely few sporadic regenerated cells. The infiltration is of the same early type as in the other cases. The hepatic parenchyma appears to have been killed with almost the rapidity of a coagulative necrosis, but the process has been protracted sufficiently for the occurrence of fatty degeneration, the complete dissolution of the majority of the cells in the centres of the lobules, the collection of a slight amount of the fat by phagocytic infiltrating cells, and for a very little regeneration of hepatic cells. The cells in the central third of the lobules were evidently the first to undergo necrosis, and their necrosis was preceded by relatively little fatty degeneration.

The *kidney* again shows severe fatty and albuminous degeneration. Hyaline, granular, and fatty cellular casts are abundant, and there is haemorrhage into tubules.

Fibres of the *myocardium* have suffered degeneration, possibly a fatty degeneration.

Case VII.

J. H. R. Aged ? Ward E 1. No. 14 Canadian General Hospital. Died ? Necropsy, March 7, 1918. (L.H.P.I., No. 588. 1917 Appendix.)

Notes received with material.

Clinical diagnoses: Jaundice. Results of arsenobillon and mercury.

Anatomical diagnosis: Acute atrophy of liver.

Macroscopic Examination.

Received from Lieut.-Col. L. W. Harrison, on March 12, 1918, the following portions of tissue, in 80 per cent. alcohol after fixation in Zenker's solution.

(1) Liver (2 × 0·8 × 0·2 cm.).
(2) Kidney (2·5 × 0·8 × 0·4 cm.).
(3) Kidney (2 × 0·5 × 0·4 cm.).
(4) Spleen (2·5 × 0·7 × 0·5 cm.).
(5) Myocardium (1·3 × 0·8 × 0·8 cm.).
(6) Lung (2 × 1 × 0·3 cm.).
(7) Pancreas (1·5 × 1·1 × 0·5 cm.).
(8) Aorta (1·5 × 0·4 × 0·15 cm.). The intimal surface was of uniform yellow colour and perfectly smooth.

Microscopic Examination.

The portion (5), myocardium, was split, and one half placed in 4 per cent. saline formaldehyde to be cut on the freezing microtome for staining in Sudan III. The remaining portions were embedded in paraffin. The paraffin sections were treated with an alcoholic solution of iodine and potassium iodide before being stained.

(1) *Liver.* The changes are almost identical with those in Case VI. There is less disintegration of the necrosed cells in the central third of the lobules. Many of the cells in the central third contain fine yellow granules, probably of lipochrome. Engorgement and haemorrhage are less. Groups of cells regenerated at the extremities of bile ductules are very rare and small. There is less cellular infiltration. Relatively large ducts are included in the section, and the lumina of these contain mucus and numerous neutrophil leucocytes.

No haemosporidia were detected. There is no pigmentation, except that described above in the more central hepatic cells.

(2) and (3) *Kidney.* The kidney is engorged, and shows the fatty and albuminous degenerations seen in previous cases. The only casts present are one or two hyaline, albuminous casts; haemorrhage is absent.

The red and white corpuscles in the blood-vessels are stained well. No haemosporidia were detected in them.

(4) *Spleen.* In the centre of many Malpighian bodies are swollen cells desquamated from the reticulum; in several Malpighian bodies there are clumps of degenerate, necrosed, and fragmented examples of these cells. The reticular strands of the pulp are narrow and contain few cells. The capillary veins contain a considerable number of free endothelial cells. There is a slight excess of neutrophil and eosinophil leucocytes, more particularly in the peripheries of the Malpighian bodies. No parasites were detected in the red corpuscles or endothelial cells. Pigment-granule cells are absent.

(5) *Myocardium.* The frozen sections were cut with difficulty, and were unsatisfactory, but small fatty granules were demonstrated by Sudan in many of the fibres.

(6) *Lung.* The arteries, veins, and capillaries are conspicuously engorged, and there are many red corpuscles within alveoli. Three short chains of Gram-positive oval cocci were found within one alveolus.

(7) *Pancreas.* Islands are numerous. There is one large irregular, and one small area of auto-digestion. Interstitial fibrosis and infiltration, and cholangitis are absent.

(8) *Aorta.* The section includes the cross-section of a branch. In neither vessel is there any abnormality.

Summary and Diagnoses.

There is an almost complete necrosis of the *liver* of the same character as in Case VI. The process of degeneration and necrosis has been even more rapid than in Case VI.

In the *kidney* there is a severe fatty degeneration associated with albuminous degeneration. The granular and epithelial casts, and the haemorrhages present in Case VI are not represented. This suggests that the injurious agent affecting the kidney has either been of less intensity or has worked for a shorter period.

In the *spleen* central necroses in Malpighian bodies, a slight proliferation of endothelial cells, and a slight excess of leucocytes indicate a slight, subacute inflammation.

The *myocardium* shows fatty degeneration.

The *lung* is greatly engorged. The presence of many red corpuscles in alveoli is probably due to rupture of capillaries by the examination of the lung, and by the removal of a portion for section.

In the *pancreas* the only abnormality is the presence of focal areas of auto-digestion.

The *aorta* has a perfectly normal appearance.

Case VIII.

Private T. A. C. Aged ? (L.H.P.I., No. 594. 1917 Appendix.)

Notes received with material.

None.

Macroscopic Examination.

The following portions of tissue, in '10 per cent. formalin', in a bottle labelled 'from No. 1 Canadian General Laboratory, Folkestone', were received on April 9 from Lt.-Col. L. W. Harrison, R.A.M.C.:

(1) Six pieces of liver (about $1 \times 0{\cdot}7 \times 0{\cdot}5$ cm. each). The cut surfaces showed a network of narrow, yellowish grey lines enclosing minute, round and polygonal, dark reddish-brown areas.

(2) Two triangular pieces of kidney; one ($1{\cdot}8 \times 1 \times 0{\cdot}4$ cm.) included a portion of medulla, the other was slightly larger and consisted of cortex alone.

(3) Two pieces of myocardium (about $1 \times 0{\cdot}7 \times 0{\cdot}5$ cm. each) of pale, clay colour.

(4) Two portions of spleen ($1 \times 0{\cdot}9 \times 0{\cdot}5$ and $1{\cdot}2 \times 0{\cdot}8 \times 0{\cdot}5$ cm.).

(5) A portion of suprarenal ($0{\cdot}8 \times 0{\cdot}6 \times 0{\cdot}5$ cm.).

(6) Two portions of lung (about $1 \times 0{\cdot}8 \times 0{\cdot}5$ cm. each). The

portions had sunk; the cut surfaces showed spongy air spaces and were of a very dark red colour.

(7) A portion of pancreas (1 × 1 × 0·5 cm.). The lobes were white, measured from 0·1 to 0·4 cm. in diameter, and were separated by narrow, grey, pliable trabeculae.

(8) A portion of small intestine (2·3 × 0·6 cm.). Within the wall a dark red area of 0·5 cm. diameter was visible.

(9) A portion of a cerebral convolution (1·2 × 1 × 0·5 cm.). The medulla was pure white; the cortex was pale yellow and measured 0·4 to 0·5 cm. in depth.

Microscopic Examination.

Portions of all the tissues were embedded in paraffin; portions of the liver, kidney, myocardium, and pancreas were cut upon the freezing microtome in order to examine for lipoids; portions of the liver, kidney, spleen, and suprarenal body were transferred to fresh 10 per cent. formaldehyde and put through Levaditi's process, with a piece of liver known to contain spirochaetes to act as a control.

(1), (2), and (3) *Liver* (Plate V; figs. 1 and 2). All the lobules have obviously decreased in size. There are minor differences in the changes in the lobules in the three different portions of liver. I shall describe the two extremes of variety under headings 'A' and 'B'.

A. In the inner third of the lobule the spaces which would normally be filled by hepatic columns contain only a few dissociated, necrosed, small hepatic cells and a few endothelial cells. Both these kinds of cell contain granules of fat, and some hepatic cells also contain granules of a yellow pigment which is stained by Sudan (lipochrome). In the outer two-thirds of the lobule, when these are examined from within outwards, the abnormally small hepatic cells are seen to become ever more numerous, to contain larger globules of fat, and more frequently to contain nuclei; the nuclei are, however, obviously degenerate. In the outer two-thirds of a few of the lobules there is at the side of a portal system a group of unbroken columns of hepatic cells (Plate V; fig. 2). When compared with the cells in the disintegrated columns, the majority of the hepatic cells here are found to be larger, more frequently to contain less degenerate nuclei, and to have a more basophil cytoplasm, in which larger fatty globules are present. In the Sudan preparations, consequently, these groups of hepatic columns are conspicuous owing to the large size and bright staining of the globules of fat therein. In the columns, however, there are also smaller, rounder, less basophil, less fatty, hepatic cells. The groups are generally rounded in shape, the columns usually being arranged in a more concentric manner than normally. Into the periphery of some of the lobules in which such groups of columns are absent, project from the ends of biliary ductules a few small club-like expansions of small hepatic cells which show little degeneration and are not fatty. The portal systems are greatly infiltrated by lymphocytes and endothelial cells. There is very little or no fibroblastic proliferation.

B. In the inner third of other lobules there are very few remnants of hepatic cells, whilst endothelial cells are here more abundant. The capillaries are wider so that the centres of the lobules appear more engorged. In the outer two-thirds of the lobules (Plate V; fig. 1) columns of hepatic cells are very rare; the hepatic cells are represented by abnormally small, rounded examples in which the nuclei usually show little or no evidence of degeneration, although the cytoplasm frequently contains small fatty granules. Endothelial cells are numerous. Groups of regenerated cells at the extremities of biliary ductules are more numerous and extend further into the lobules. Great cellular infiltration extends from the portal systems into the outer third of the lobule. Among the infiltrating cells leucocytes are exceptional. There is a proliferation of fibroblasts round the periphery of the portal systems and round the central veins; an increase in collagen fibrils is not, however, recognizable.

The bile ducts and ductules in A and B contain a few neutrophil leucocytes. The larger bile ducts contain, in addition, a little mucus.

No haemosporidia were detected. Lipochrome granules have been mentioned above. A precipitate of pigment granules is also present where the tissue is engorged, and has the character of the precipitate formed by the action of formaldehyde on haemoglobin. There is no accumulation of melanin.

(4) *Kidney.* The kidney shows a severe fatty and albuminous degeneration such as has been described in other cases. Numerous cells in the first convoluted tubules are necrosed, the entire epithelium in many sections of these tubules containing no nuclei. Many of the collecting tubules in the pyramids of Ferrein contain masses of deeply haematoxylinophil substance. Many of these masses are composed of haematoxylinophil spheres, and in some such spheres nuclei are present. The masses are evidently formed by the aggregation and confluence of altered desquamated cells. The appearances suggested calcareous impregnation, and some of the masses gave a faint Prussian blue reaction, but the substance was not stained by silver nitrate.

There are also hyaline, granular, and epithelial casts. There is no active inflammatory reaction.

(5) and (6) *Myocardium.* In paraffin sections fine vacuolation of many fibres is seen. In Sudan preparations the great majority of the fibres contain fat. The fat is usually in the form of minute, rarely of large, globules. The fatty degeneration is distributed throughout the myocardium in large patches.

(7) *Spleen.* In a few of the Malpighian bodies there are central necroses such as have been described in preceding cases. The capillary veins are moderately engorged. The reticular strands contain numerous, free, endothelial cells, a large number of which are typical plasma cells (Unna-Pappenheim stain). There is a slight excess of neutrophil polymorphonuclear leucocytes in the capillaries and reticular strands. Peripheral nodular hyperchromatosis and complete fragmentation of nuclei are frequent. The red corpuscles have been improperly fixed in the tissue furthest from the capsular surface.

There is a considerable deposit of formaldehyde pigment; it is most abundant where the red corpuscles are disintegrated. The Prussian-blue reaction demonstrates one group of cells containing granules of iron-pigment in the wall of one large vein.

No malarial parasites were detected.

(8) *Suprarenal body.* The section includes both cortex and medulla. There is nuclear degeneration in a few cortical cells, especially in the fascicular zone.

No malarial parasites were detected in the vessels.

(9) *Lung.* The majority of the air spaces contain numerous red corpuscles and a few polymorphonuclear leucocytes. A few alveoli contain a thin, coagulated, albuminous substance. There is a slight infiltration of the walls of the bronchioles with neutrophil leucocytes. In the alveoli are numerous Gram-positive, rounded cocci, diplococci and streptococci, less numerous Gram-negative, slender bacilli, and conspicuously large Gram-positive cocci, diplococci and streptococci in short chains. These organisms form masses in bronchioles and alveolar bronchioles. Brown-yellow granules, apparently of formaldehyde pigment, are numerous throughout the section; the leucocytes are loaded therewith. Much of the interstitial tissue is stained a diffuse brown-yellow. There is no iron pigment. The red corpuscles are not laked.

(10) *Pancreas.* The glandular tissue throughout has undergone a variable amount of autolysis, and there are patches of lipolysis in the lipomatous septa and capsule. There is no evidence of acute or chronic inflammation. The cytoplasm of the cells of the islands is filled by small granules which are stained by Sudan: Sudanophil globules are also present round the nuclei of a few of the cells of the acini.

(11) *Jejunum.* The summits of the villi have undergone post-mortem necrosis. The blood is laked. There is a large haemorrhage in the submucosa and muscularis. Throughout the section, and most abundant in the tissues round the haemorrhage, are granules of formaldehyde precipitate. There is no inflammatory reaction.

(12) *Cerebral cortex.* The pyramidal cells are of angular shape and have central nuclei, but the nuclei are usually pyknotic and the cytoplasm is devoid of Nissl's granules. A few red corpuscles lie in the meshes of the lepto-meninges. The vessels in several slides were searched in vain for malarial parasites.

(13–16) *In the Levaditi preparations of the liver, spleen, kidney, and suprarenal body* no spirochaetes were found; numerous spirochaetes were seen in the portion of syphilitic liver employed as a control.

Summary and Diagnoses.

The liver. The changes in the liver are essentially similar to those in the preceding cases. There are slight differences in the lesions in different parts of the tissue examined. Where the changes are such as I have described under 'B', they resemble closely those found in Case III. There has been almost complete

destruction of the whole lobule. Very few of the original hepatic cells remain. Young hepatic cells have been formed by proliferation of undamaged cells in the columns in the outer parts of the lobules, and by proliferation of hepatic cells at the extremities of biliary ductules. The majority of the cells regenerated in the site of the columns have undergone fatty degeneration. That the process has been of longer duration than in Case III is shown by there being more fibroblastic proliferation in the periphery of portal systems and round central veins, and by the infiltration containing very few leucocytes.

In the portions affected by the changes described under the heading 'A', the lobules have undergone less initial destruction. Their centres had undergone severe degeneration and necrosis, but there had been less destruction in the outer parts of the lobules: whole groups of the original columns had escaped dissolution in the outer two-thirds of some of the lobules. These groups of columns have become rounded in shape; the columns within them tend to have a concentric direction in place of the normal radial; among the cells of ordinary size are small, rounded, less basophil, less fatty, young hepatic cells (Plate V; fig. 2). These changes indicate regeneration in the groups of columns. The destruction of the parenchyma in the periphery of the lobules having been less, there has been less regeneration from the extremities of biliary ductules. The original destruction of the parenchyma having been less, there has been less proliferation of granulation tissue. Such original cells as remain in the groups of columns have undergone severe fatty degeneration. That the fatty degeneration is greater in these cells than in any others is explained by the general observation that the more rapid the degeneration and necrosis of a cell the less is the development of fatty degeneration.

In this liver there is evidence, therefore, that some lobules have originally been destroyed more extensively than others. In both cases destruction has been greatest in the centres of the lobules. In both there has been regeneration, and in both degeneration has subsequently affected the regenerated cells. The processes have been of longer duration than in the other cases, doubtless because the initial destruction has in places been less.

The cholangitis present in the preceding cases is represented here.

The *kidney* shows the severe fatty and albuminous degenerations seen in the preceding cases. Many of the collecting tubules in the pyramids of Ferrein contain masses of a haematoxylinophil substance formed by the confluence of cells which have been transformed into spheres of this substance. Similar spheres were seen in the kidney of Case V. The appearances, including a positive Prussian-blue reaction, suggested calcification, but the substance was not stained by silver nitrate.

The *myocardium* shows severe fatty degeneration.

The changes in the *spleen* are those of a slight subacute inflammation.

The *suprarenal body* is normal.

The *lung* shows haemorrhage, early acute inflammation of the bronchial walls, and exudation of serum into a few alveoli. Inflammatory infiltration of the air passages, however, is absent. There are numerous bacteria of different kinds throughout, and these bacteria form masses within bronchioles and alveolar bronchioles. The lung is the site of a very early broncho-pneumonia, apparently an agonal aspiration broncho-pneumonia.

The *pancreas* is the site of an auto-digestion so extensive that it is without doubt partly, if not entirely, a post-mortem phenomenon. The cells of the islands contain fatty granules, and similar granules are present round the nuclei of a few of the cells of the acini; according to my experience, these appearances in Sudan preparations cannot be regarded as of pathological significance.

In the *jejunum* there is a large haemorrhage into the muscularis and submucosa, unaccompanied by evidence of inflammation.

In the *cerebral cortex* the pyramidal cells show a tigrolysis and nuclear degeneration to which no pathological significance can be attached in the absence of evidence that post-mortem changes had not had time to occur before fixation.

Haemosporidia were not detected, and there are no accumulations of pigment such as are found in severe malarial infections.

Spirochaetes were not detected in the liver, spleen, kidney and suprarenal body.

General Summary of Cases, and Conclusion.

The material examined was obtained from eight patients. It came in two cases from Cherry Hinton Military Hospital, Cambridge in five from No. 14 Canadian General Hospital, and in one from No. 1 Canadian General Laboratory, Folkestone.

The material comprised portions of the liver in all eight cases, of the kidney in six cases, of the spleen in five, of the myocardium and of the lung in four, of the pancreas in three, of the suprarenal body in two, of the jejunum, appendix, aorta, and cerebral cortex in one case.

The liver was in all cases the site of a severe pathological lesion, of a lesion more severe than that in any other organ. Moreover, in no other organ were changes found to which those in the liver could be regarded as secondary.

There is a remarkable resemblance between the lesions in the different livers and in different portions of the same liver. This similarity encourages the belief that it is justifiable to base conclusions on an examination limited to so small a number of cases. There are, however, slight differences between the lesions in different cases, and in different portions from some individual cases. In the summaries given above at the end of each case I have attempted not only to elucidate the nature of the pathological process but to determine its course and to make a comparative estimation of its duration in each case.

In this attempt I have laboured under the serious disadvantages,

first of never having seen the whole organ, and secondly of not having witnessed a necropsy. It is obvious that the course of the process cannot be ascertained by comparison of pieces taken from the same and different livers unless such pieces are representative of the condition present throughout the organs. It would appear justifiable, however, to conclude that the pieces sent are fair representatives of the condition present throughout each liver, because there is so little variation even in the microscopic appearances in different cases and in multiple pieces taken from individual livers; several pieces were removed from the liver in six cases, and such pieces were doubtless selected so as to include any recognizable variations. There certainly are differences in the histological picture in different portions taken from the same liver, but these are very slight; they are most pronounced in Case VIII (Plate V; figs. 1 and 2). Ignorance of all the conditions present in the bodies at necropsy makes it impossible to be certain that the condition found in the liver was the actual cause of death in every case. If death was due to a complication, and no evidence of that complication was to be found in the tissue taken for microscopic examination, whilst the presence of post-mortem autolysis in the liver rendered the determination of the extent of ante-mortem necrosis impossible, erroneous conclusions would be drawn concerning the extent and rapidity of the hepatic lesion. Fortunately, post-mortem autolysis has seldom been so great as to make this source of error of moment, and I presume that the portions of other tissues sent for examination represent all other lesions recognized or suspected in the body.

The pathological process in the liver can be described, in general, as follows.

The liver is in every case the seat of severe degeneration and necrosis. The centres of the lobules have been most vulnerable; they are always severely affected, and such variations as are found in the histological pictures depend upon the extent to which, and the rapidity with which, the peripheral zones have also been affected. In the degeneration and necrosis fatty degeneration plays a considerable rôle. It is slight where necrosis has been rapid, and greatest in cells which have survived longest; thus, it is slight in the centres of the lobules, and attains its greatest magnitude in remnants of the original hepatic columns in the cases in which the progress of the pathological processes has been slowest. The formation of lipochrome granules is usually conspicuous in the cells of the more central portions of the lobules; it also occurs within the epithelium of biliary ductules.

The rapidity of the destructive process appears to have varied in different cases. The criteria employed for the determination of the duration of the process have been mentioned in the summary of Case I (pp. 9–10); in the subsequent summaries the relative rapidities of the process have been compared. In Case II the liver appears to have undergone the most rapid necrosis. The hepatic columns in the outer two-thirds of the lobules have been killed almost *en masse*. There is relatively little fatty degeneration and there is no evidence of regenerative proliferation. In Cases I, VI, and VII, the columns

in the outer two-thirds of the lobules have died almost as rapidly, but there has been time for more fatty degeneration, for a trace of replacement by proliferation of cells in the hepatic columns, and for a slight proliferation of cells at the end of biliary ductules. In Case IV there has been more replacement of the cells in the original columns in the outer two-thirds of the lobules by young cells; the destruction of the columns has been more gradual, allowing a considerable number of less damaged cells to proliferate and replace those necrosed. Proliferation of cells at the end of biliary ductules is also greater. There is a very slight proliferation of interstitial tissue. In Cases III and V the original columns are represented only by a few small remnants, chiefly in the outer third of the lobules. The most numerous cells in the site of the columns in the outer two-thirds of the lobules are young, proliferated hepatic cells. These cells are not sufficient in number to fill the spaces which should be occupied by columns; they are, indeed, few in number, and are most numerous in the outer third of the lobules. The proliferation of cells at the end of biliary ductules is now greater. Fibroblastic proliferation, about the portal systems and central veins, is more pronounced. The cellular infiltration is still of an early type, containing many leucocytes.

In the above cases there was very little difference between the affections of different lobules. In Case VIII differences in different portions of the liver were more marked. In parts the changes were very similar to those in Case III, only fibroblastic proliferation was slightly greater, and the infiltration was of slightly older type. In other parts groups of the original hepatic columns in the periphery of the lobules had persisted, and had undergone a slight regeneration. The pathological process appears to have endured for a longer time in this case than in any of the others, doubtless because the initial destruction had been more limited, as is shown by the persistence of, and regeneration in, columns of hepatic cells in the periphery of some of the lobules.

A comparison of the cases shows, therefore, that in some the destructive process has been more rapid than in others. In those in which it had been more chronic there had been time for regenerative proliferation of cells in the hepatic columns, and of cells at the extremities of bile-ductules. Now, in some degenerations of the liver the process of destruction and replacement of destroyed cells may take place very gradually, and may be accompanied by no fibroblastic proliferation and no appreciable infiltration. In typical examples of this gradual destruction and replacement there are no deficiencies in the hepatic columns. I do not know how the duration of such a process can be measured. In the present cases any replacement of necrosed cells is associated with so great a deficiency of cells throughout the columns, that it would appear justifiable to consider that in all cases the destruction was such as would even in the earlier stages stimulate infiltration and fibroblastic proliferation. The whole duration of the process can, therefore, be estimated by the character of the infiltration and the degree of fibroblastic

proliferation. According to these criteria the destructive process has been very rapid in action, even in the most chronic examples.

This conclusion is supported by the amount of regeneration at the terminations of biliary ductules; even in the most chronic cases it is small, and corresponds to that which usually accompanies infiltrations of a similarly early type, and interstitial proliferation of such early development. The regenerative proliferation of cells of the hepatic columns is, however, greater than I have seen it in livers in which the other changes have been similar. This peculiarity is difficult to explain. It probably indicates that, in the livers in which it is present, there have been short intermissions in the action of a damaging agent of great intensity.

Where cells have been formed by regeneration many have undergone degeneration. This degeneration may be the result of interference with the circulation such as might well be caused by haemorrhage and capillary dilatation in the centres of the lobules. In view of the changes in other organs it would appear much more likely to be the result of a continuance or renewal of the action of the damaging agent.

The most important point to be determined is the *nature of the damaging agent*. No *bacteria* were identified in the liver except in Case II. In this case there was very severe post-mortem degeneration, and the bacteria, Gram-positive bacilli, had the appearance and distribution of bacteria which are frequently found when the removal and fixation of tissues have been delayed. Similar bacilli were found in the pancreas of Case IV. The only bacterial infections found were very early broncho-pneumonias in Cases IV, V, and VIII. These pneumonias were evidently terminal complications. There was, therefore, no evidence of any bacterial infection to which the hepatic lesion can be ascribed. Several portions of the liver in Cases I, II, and VIII, and of the kidney, spleen, and suprarenal body in Case VIII were put through Levaditi's staining process. No *spirochaetes* were found, although spirochaetes were clearly demonstrated in portions of a syphilitic liver employed to control the stain. I was informed that *malarial parasites* had been detected in the tissues of some of the cases of death following the administration of salvarsan. I therefore paid particular attention to a search for evidence of their presence. I failed to find haemosporidia. In some cases the condition of the material was such that the presence of these parasites would not have been demonstrated. In no case, however, were there accumulations of melanin. Iron pigment was only detected in one small group of cells in the wall of a vein in the spleen of Case VIII.

In the examination, therefore, of these eight cases no evidence was obtained that the damaging agent was connected with the growth of organisms within the body. It is obvious that this negative evidence is not conclusive. The number of cases examined is not great, and much of the material was in a poor condition of preservation. It is an established fact that spirochaetes may not be detected, even by the most careful examination, in the tissues of patients who have died as the result of a proven infection by the

spirochaete of haemorrhagic jaundice. According to Pick (*Berliner klinische Wochenschrift*, 7. Mai 1917, p. 451), Schmorl, whose persistence in investigation and remarkable technical skill are proverbial, failed to find spirochaetes in his material, Beitzke was successful in two cases only, and Herxheimer in one. Further, the methods adopted in the above examination could not demonstrate an infection by a filterable virus. It can, however, be said that the hepatic lesion differed from those known to be caused by organisms, either in the uniform severity of the destruction of the liver or in histological details. My experience of the action of spirochaetes upon the tissues is limited to that of the *Spirochaeta pallida*. Lesions characteristic of syphilitic infection were found neither in the liver nor in any of the other tissues examined. I have not had an opportunity of examining material from cases of spirochaetosis ictero-haemorrhagica. From the description of the hepatic changes in this infection given by Pick (*loc. cit.*) severe destruction appears to be rare; a large number of young hepatic cells, and hepatic cells containing karyokinetic figures appear usually to be the chief evidence of destruction. Fatty degeneration is absent or is slight. Sir Bertrand Dawson (*The Lancet*, November 2, 1918, p. 575) mentions one case in which complete destruction of parenchyma was found, but does not give the extent of that destruction. He quotes Hart as having found that the disease falls with varying force upon the liver, the changes showing gradations from the slightest deviation from normal structure to an extreme disorganization indistinguishable from acute yellow atrophy. In constancy of severity, therefore, and in the prominence of fatty degeneration, the hepatic lesions in the salvarsan cases contrast sharply with those caused by the spirochaete of haemorrhagic jaundice. In fatal cases of yellow fever examined by me[1] the hepatic lesion was very extensive, affected the liver uniformly, and showed little difference in degree in different cases. But the histological details differed conspicuously from those found in the salvarsan cases. Fatty degeneration was more pronounced; necrosis was less; actual loss of parenchyma and disturbance of the continuity of the hepatic columns were very slight, cellular infiltration was much less, and free iron was deposited in many hepatic cells.

On the other hand, the lesions in the salvarsan cases resemble very closely those found in cases in which there can be little doubt that the essential damaging agent is an exogenous chemical poison, for instance the lesions attributed to phosphorus, trinitrotoluene, and tetrachlorethane. The lesions, it is true, are not exactly like those caused by these three poisons. In a lesion of equal intensity and distribution caused by phosphorus, fatty degeneration is much more conspicuous. In trinitrotoluene poisoning the share in the degeneration and necrosis taken by fatty degeneration is very similar to that in the present cases. A characteristic, however, of all cases of trinitrotoluene poisoning which I have examined[2] has

[1] Yellow Fever Commission (West Africa). *Reports on Questions connected with the Investigation of Non-Malarial Fevers in West Africa*, 1915, vol. i, p. 196 and p. 219.

[2] A summary of the histological changes observed in my earlier cases is given in the *Proc. Roy. Soc. Med.*, General Reports, 1917, 10, 47.

been the great difference in the extent to which different portions of the liver have suffered. In certain portions of the liver the parenchyma of whole lobules has been entirely destroyed, but even when the destructive process has been most acute and most widespread, there have also been areas in which necrosis has been confined to the centres of lobules (Plate VI; figs. 1 and 2). Thus to the naked eye sunken areas in which the parenchyma was entirely destroyed contrasted conspicuously with raised areas in which the lobular pattern was disturbed only by a variable degree of central necrosis and of interstitial proliferation about portal systems and central veins. This characteristic was also present in the only case of tetrachlorethane poisoning from which I have obtained material, and in a case in which acetone was suspected to be the poison. Another characteristic of the material from trinitrotoluene workers was the great variation in the age of the lesions in different cases; thus, I have obtained examples of all stages of interstitial reaction up to the development of dense fibrous tissue containing stout, elastic fibres of new formation. This second characteristic obviously depended upon the first. The focal variations in the intensity of the destructive process left intact quantities of parenchyma sufficient in some cases to delay death, in others to allow the liver to recover completely; the oldest lesions were found as focal hepatic scars in cases in which the liver had evidently recovered completely, and death was due to a subsequent aplastic anaemia.

These characteristics of the lesions caused by trinitrotoluene do not prove that there is any essential difference in the actions of trinitrotoluene and the damaging agent in the salvarsan cases. They merely indicate that trinitrotoluene is a less intense poison, or reaches the liver in smaller doses. In phosphorus poisoning death may occur within a few days; the whole liver is then found to be uniformly and severely affected. In other cases of phosphorus poisoning the fatal issue is delayed; a variable amount of the parenchyma is then found to have escaped damage. There can be no doubt that these differences in the extent of the lesions depend upon differences in the doses of the phosphorus, or, possibly, the susceptibility of the livers. In trinitrotoluene poisoning a greater or less part of the liver invariably escaped destruction, and the more vulnerable portions were alone destroyed. The more vulnerable portions were those furnished with the poorest supply of oxygenated blood. Thus, in the acuter cases, there was in almost all lobules destruction of the portions furthest from the oxygenated blood supplied by the portal systems, that is to say, the centres of the lobules and lines uniting the centres of neighbouring lobules through points equidistant from adjacent portal systems. Whole lobules were destroyed in positions in the organ corresponding to those in which the greatest destruction is caused by venous congestion in chronic heart-failure. In more chronic cases, and cases in which there was complete recovery, destruction was very limited. In all the eight cases of death after salvarsan the poison was evidently of much greater virulence, and almost the whole of every lobule was destroyed. Severe destruction of the liver being much more

uniform, death occurred with greater and more uniform rapidity, and, consequently, detectable differences in the duration of the destructive process were much less. Mention has been made of a peculiarity in the salvarsan lesions: severe, apparently rapid, destruction was associated in many cases with considerable regenerative proliferation of hepatic cells in the site of the disintegrated columns. I have suggested that this is the result of short intermissions in the action of a virulent toxin.

In trinitrotoluene poisoning strong evidence was obtained that the destructive process was progressive, and might progress for long periods after cessation of work with the poison; this is probably explained by persistence of the poison in the tissues. In the salvarsan cases regenerated cells underwent degenerations similar to those which affected the original tissue. These degenerations might possibly be the consequence of the severe disturbance in the anatomy of the liver. But when the degeneration in other organs is considered, there can be little doubt that they were due to persistence, or to repetitions, of the damaging agent.

The hepatic lesion in the salvarsan cases bears, therefore, a strong resemblance to those attributed to exogenous poisons. It differs from that of severe phosphorus poisoning in that fatty degeneration plays a smaller rôle. It resembles more closely the lesions caused by trinitrotoluene and tetrachlorethane, but differs in the greater virulence or dosage of the destructive agent, and in the constancy of that virulence or dosage.

Jaundice and cholangitis. No accumulations of bile-pigment were found in the sections of the liver. In the cases of fatal trinitrotoluene poisoning, in the lobules in which the hepatic columns had not been disintegrated, many of the biliary canaliculi were distended by bile. Inasmuch as the hepatic columns were almost entirely disintegrated in all save the acutest of the salvarsan cases, distension of canaliculi by bile was seldom possible.

The biliary ductules, and their extensions as pseudobile canaliculi, did not contain bile, but contained occasional neutrophil polymorphonuclear leucocytes. In Case II the ducts contained a few neutrophil leucocytes. In Cases I, III, VII, and VIII large bile-ducts contained mucus and neutrophil leucocytes. In Cases I and VII the leucocytes were present in sufficient number to justify the term 'muco-purulent cholangitis'. In Case VI no large bile-ducts were present in the sections.

Of eight cases of fatal poisoning by trinitrotoluene, in one there was catarrhal desquamation of the epithelium of the bile-ducts, and in another the ducts contained an albuminous substance and a few neutrophil leucocytes. In the earlier cases of trinitrotoluene poisoning examined by me the content of the gall-bladder suggested inflammation. I therefore examined the wall of the gall-bladder microscopically in three subsequent cases; mucous catarrh was present in one, and a subacute inflammatory infiltration of the wall in the other two. A subacute inflammatory infiltration of the wall of the gall-bladder was present also in a case of similar destruction of the liver which occurred in a worker with acetone. There is,

therefore, some evidence that cholangitis and cholecystitis are associated with destructions of the liver which are caused by the action of exogenous poisons. It is possible that the poisons are excreted from the liver along the bile-ducts, and excite inflammation therein, or render them vulnerable to infection from the duodenum. The gall-bladder was not included in the material sent for examination in the salvarsan cases.

The kidney was available for examination in six cases, and in all showed a severe parenchymatous degeneration. In this degeneration fatty degeneration was so conspicuous that it could be diagnosed in tissue embedded in paraffin. Catarrhal desquamation, and hyaline, granular, and epithelial casts were also present. In Case VI there were a few haemorrhages into tubules; in Case IV there was a large haemorrhage into the interlobar connective tissue. In two cases, V and VIII, there was a peculiar haematoxylinophil degeneration of cells of the collecting tubules in the pyramids of Ferrein; the affected cells were swollen and formed transitions to spherular bodies and to concretions formed by the confluence of these. The appearances suggested calcareous impregnation, which is not infrequent in the human kidney, but Kossa's reaction for calcium proved negative. In Case VIII the concretions gave a very faint Prussian-blue reaction, and inasmuch as calcareous portions of tissue assimilate any iron in fixatives and reagents, a trace of calcium may have been present. It is possibly of significance that a haematoxylinophil necrosis of vascular endothelium was present at the site of haemorrhage in the appendix, and here, too, the reaction for calcium failed.

In no kidney was there an active inflammatory reaction. There was, therefore, no true nephritis.

The changes in the kidney are such as might result from an infection. They are also such as are very constantly found in cases of destruction of the liver by exogenous poisons. In acute phosphorus poisoning there is severe fatty degeneration of the kidney. The frequency with which fatty degeneration of the kidney is associated with acute destruction of the liver, for instance, in cases of acute atrophy of unknown origin, suggests that the renal degeneration may be the sequel of the changes in the liver. In the cases of trinitrotoluene poisoning, however, the amount of fatty degeneration in the kidney corresponded very closely to the estimated activity of the poison upon the liver at the time of death. The simpler explanation, therefore, that the lesion in the kidney is due to the direct action of the poison which damages the liver, would appear to be correct. In the cases of trinitrotoluene poisoning, in which the poison was estimated to be active at the time of death, the changes in the kidney resembled those of the salvarsan cases very closely. A true acute nephritis was present in a case in which Professor Moore produced, by experimental administration of trinitrotoluene, lesions in the liver of a rabbit very similar to those found in man. I have been informed that calcification, and therefore necrosis, is frequently found in the kidneys of rabbits which have died a short time after the injection of toxic salvarsan preparations.

The large haemorrhage into the interlobar tissue of the kidney in Case IV was doubtless similar in origin to the haemorrhages in the intestine. The haemorrhages into the tubules in Case VI may have had a like origin.

The myocardium was examined in four cases. In two cases sections could not be prepared for the demonstration of fat. Vacuolation in the paraffin sections, however, suggested fatty degeneration. In the two cases in which Sudan preparations could be made, fatty degeneration was present. There was no active inflammatory reaction.

Fatty degeneration of the myocardium is frequently found in cases of acute destruction of the liver by exogenous poisons. Extensive fatty degeneration was present in the myocardium of cases of acute poisoning by trinitrotoluene.

The spleen was available in five cases. In one case, IV, the spleen showed the changes of a 'red septic spleen' of mild degree. A very few Gram-positive diplococci were found in the organ, and pneumococcal broncho-pneumonia was present in the sections of lung. Inasmuch as this was the only true septic spleen in the series, there can be little doubt that it was due to the broncho-pneumonia.

In the remaining four cases there were central necroses in the Malpighian bodies, and evidence of a slight subacute inflammatory reaction in the pulp. These changes may be evidence of infection, but I found similar changes in the spleen in cases of trinitrotoluene poisoning. They have also been present in other cases of destruction of the liver attributed to exogenous poisons. The changes would appear to depend upon the disturbances of hepatic function, and are possibly the early manifestations of the changes which are found in the enlarged spleens associated so constantly with obliteration of the portal or splenic veins, or with chronic destruction and fibrosis of the liver.

The pancreas was examined in three cases. In Case VIII there was a general digestion of the organ, much, if not all, of which doubtless occurred after death. In this case alone was it possible to make Sudan preparations. The cells of the islands contained numerous Sudanophil granules, and fatty globules were present round the nuclei in a few cells of the acini. I have found a similar picture so constantly in Sudan preparations of the pancreas that it would appear to have no pathological significance.

In Cases IV and VII there were focal areas of digestion of the glandular and lipomatous tissues. It is possible that these were areas of post-mortem digestion; putrefactive bacilli were present in Case IV. Inasmuch, however, as the remainder of the gland was well stained in both cases it is more likely that the digestion occurred before death. I examined the pancreas in six cases of acute poisoning by trinitrotoluene. Digestion was present in all. In three cases it was universal, being in two associated with infiltration by putrefactive bacteria. In the three other cases there were numerous focal areas of digestion. The tissues were in no case perfectly fresh when fixed. I was therefore inclined to regard

the digestion as a post-mortem phenomenon. But in a subsequent case of very similar acute destruction of the liver, supposed to be caused by acetone, the pancreas removed soon after death and fixed in excellent condition, also contained focal areas of digestion. In this case no spirochaetes or other organisms could be detected. It was therefore impossible to ascribe the digestion in all cases to post-mortem changes, and I came to consider it probable that ante-mortem digestion was very constantly associated with acute destruction of the liver by poisons. So little definite is known concerning the causes of ante-mortem pancreatic digestion that it would be idle to discuss them here. But the commonest associated conditions are certainly the presence of calculi and inflammation in the gall-bladder. Inasmuch, therefore, as cholecystitis was found in all trinitrotoluene cases in which the gall-bladder was examined, and was found in the case of supposed acetone poisoning, whilst a cholangitis was found in so many of the salvarsan cases, it is possible that inflammation of the biliary passages was the cause of the pancreatic digestion. Evidence has been given above that exogenous poisons which destroy the liver may cause directly, or indirectly, inflammation in the biliary passages. In none, however, of the salvarsan cases, or the other cases quoted, was there histological evidence of inflammation within the pancreatic ducts. Further, there was no evidence of inflammation within the pancreatic tissue except in Case IV, above, and in this case the inflammation was chronic, being manifested by atrophy, fibrosis, and slight lymphocytic infiltration of a few lobules in the periphery of the gland.

The Intestine. A portion of the *appendix* was sent for examination in Case III and of the *jejunum* in Case VIII. In both appendix and jejunum there was haemorrhage into the wall of the gut. In neither specimen was there evidence of inflammation. In the lumen of the appendix, and in the cavities which had been torn in the wall by the haemorrhage and which communicated with the lumen, were very numerous bacteria of different kinds; a few similar bacteria were also present within vessels. In the absence of any inflammatory reaction it is not possible to ascribe the haemorrhage to the action of these bacteria.

The haemorrhages into the intestine were not the result of inflammation. They may have been caused either by toxins resulting from hepatic insufficiency, or by the direct action of the poison which destroyed the liver. Haemorrhages are frequently associated with destruction of the liver, whether the destruction be the result of infection or of exogenous poisons. Haemorrhages, particularly subperitoneal haemorrhages, are numerous in cases of poisoning by trinitrotoluene. I have been informed that haemorrhages in the intestine are frequently found in animals which have died a very short time after injection of toxic salvarsan. The peculiar, haematoxylinophil necrosis of endothelial cells lining the vessels in the appendix suggested that histological evidence was present of the manner in which the toxin caused haemorrhage. This necrosis was not seen, however, in the jejunum.

Material was not available for specific staining for fat, so that it is not possible to say whether or not fatty degeneration of the endothelium was present.

The haemorrhage into the interlobar interstitial tissue of the kidney in Case IV was doubtless similar in origin to the haemorrhages in the intestine; the haemorrhages into the tubules of the kidney in Case VI may have had a like cause.

Portions of *lung* were examined in four cases. In Case V there was a very early pneumococcal pneumonia, in Case IV a more fully developed, acute, pneumococcal broncho-pneumonia, and in Case VIII an early broncho-pneumonia in which the variety and number of bacteria indicated infection by aspiration or deglutition. In Case VII great engorgement was associated with a considerable number of red corpuscles in the alveoli. The number of corpuscles in the alveoli was less than would be expected in haemorrhage, so that the escape of blood was more probably due to mechanical extravasation in the manipulation of the tissue at the necropsy. In the lungs, therefore, no pathological changes were found other than those which very frequently complicate the later stages of severe illness.

In the one specimen of *cerebral cortex* (Case VIII) there were tigrolysis and slight pyknosis in the bodies of the neurons, but no pathological significance can be attached to these changes in the absence of evidence that post-mortem degeneration had not had time to occur before fixation.

In the *suprarenal body* in Cases IV and VIII and in the *aorta* in Case VII no pathological changes were detected.

Conclusion. The chief lesion is a very severe destruction of the liver caused by the action, probably in the more chronic cases the intermittent action (p. 35), of a toxin of great toxicity or of large dosage. The hepatic lesion is accompanied by a severe, largely fatty, parenchymatous degeneration of the kidneys, a fatty degeneration of the myocardium, and a slight, subacute inflammation of the spleen. Cholangitis and auto-digestion of the pancreas are frequent. There may be haemorrhages into the intestine, the interlobar tissue of the kidney, and the renal tubules. In the examination of these eight cases no micro-organisms were detected, except bacteria in terminal infections. This negative finding obviously does not exclude the possibility of the toxin having been the product of an infection. The lesion in the liver differs, however, from those known to be caused by infection, in the greater constancy of the severity of destruction or in histological details. Moreover, the hepatic lesion is very similar to those attributed to the action of exogenous poisons. It resembles very closely that caused by trinitrotoluene, but shows differences which are adequately explained by the assumption that in the salvarsan cases the poison was constantly of greater virulence or administered in greater dosage. Further, the pathological changes in other organs may all, it is known, be caused by exogenous poisons; all occur in acute poisoning by trinitrotoluene, the majority occurring constantly.

The above conclusion does not pretend to be more than provisional. The arguments rest upon observations which require further confirmation. To take only one example, great stress has been laid upon the severity, and the uniformity of the severity, of the hepatic lesions. Had eight complete livers in a fresh condition been examined, considerable reliance could be placed in the uniformity of the changes. But, small pieces of liver have alone been available for examination, and in some cases the pieces had undergone severe post-mortem change. The occurrence of a single example of slight, focal destruction of the liver would necessitate considerable modification of certain of my inductions.

PLATE I

FIGURE 1

Liver. Case I. Weigert's iron-haematoxylin and van Gieson's mixture. (× 136.)

Includes central vein and two portal systems of a lobule. Fibres which were stained red are black. Cells containing lipochrome granules are stippled black.

FIGURE 2

Liver. Case I. Haematoxylin and eosine. (× 43·5.)

From area of slightly greater parenchymatous destruction, showing proliferation of biliary ductules.

PLATE I

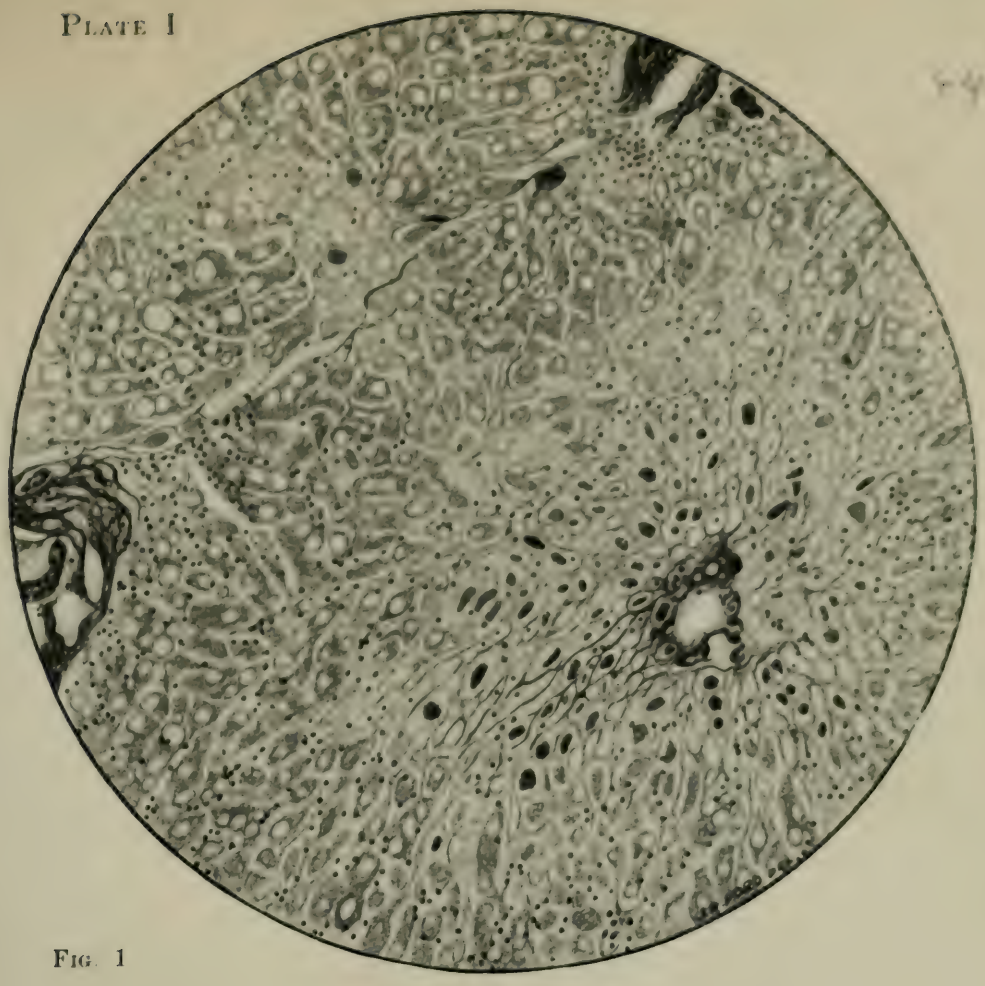


FIG. 1

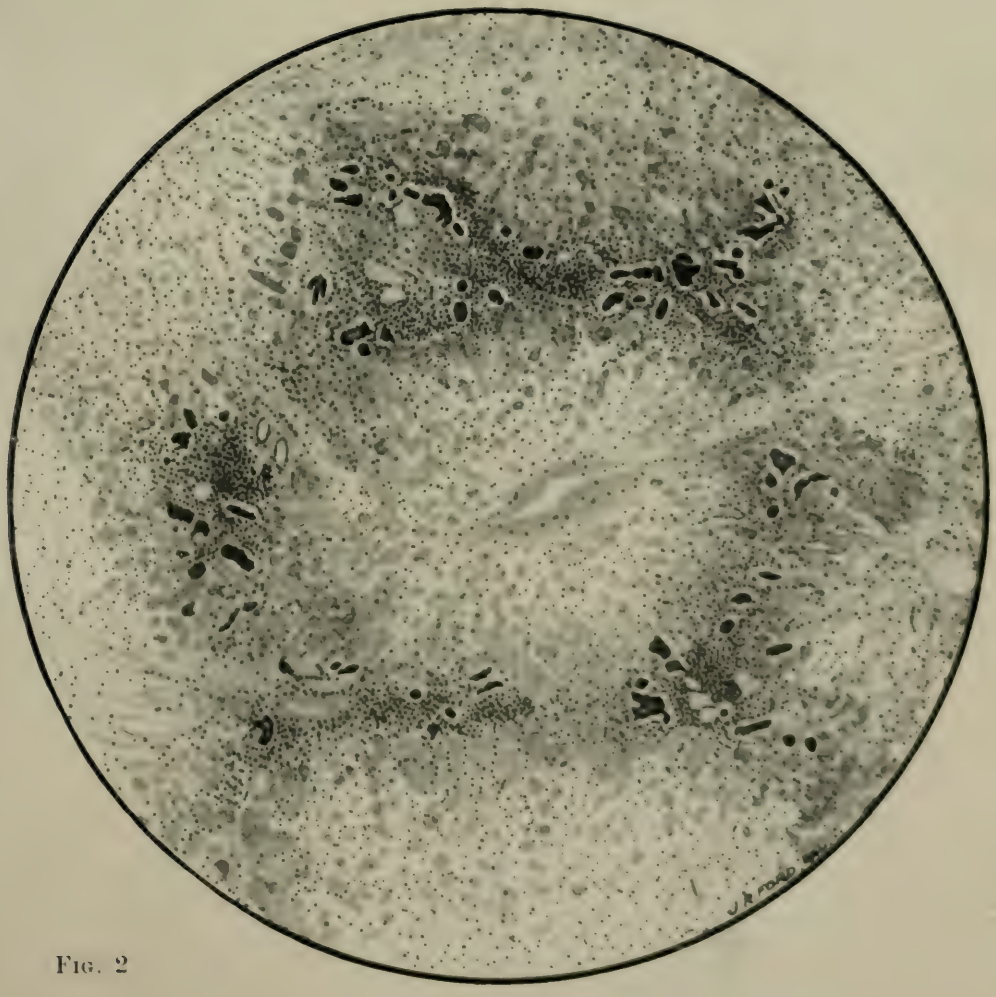


FIG. 2

PLATE II

FIGURE 1

Liver. Case I. Haematoxylin and eosine. (× 335.)
Bile duct containing mucus and neutrophil leucocytes.

FIGURE 2

Kidney. Case III. Haematoxylin and eosine. (× 230.)
Demonstrates fatty degeneration as shown in paraffin sections. Compare Plate III, figure 2.

PLATE II

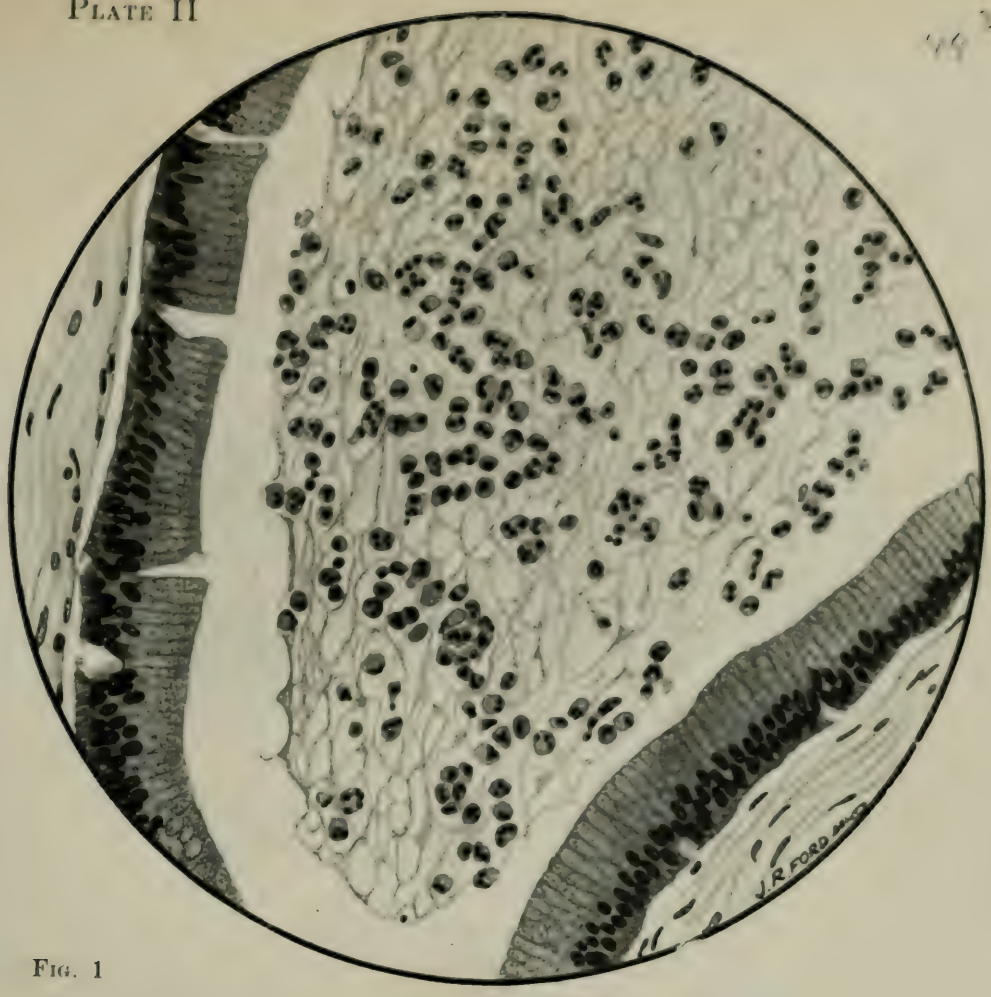


FIG. 1



FIG. 2

PLATE III

FIGURE 1

Liver. Case I. Sudan III and haematoxylin. (×76.)

FIGURE 2

Kidney. Case VI. Sudan III and haematoxylin. (255.)

Plate III.

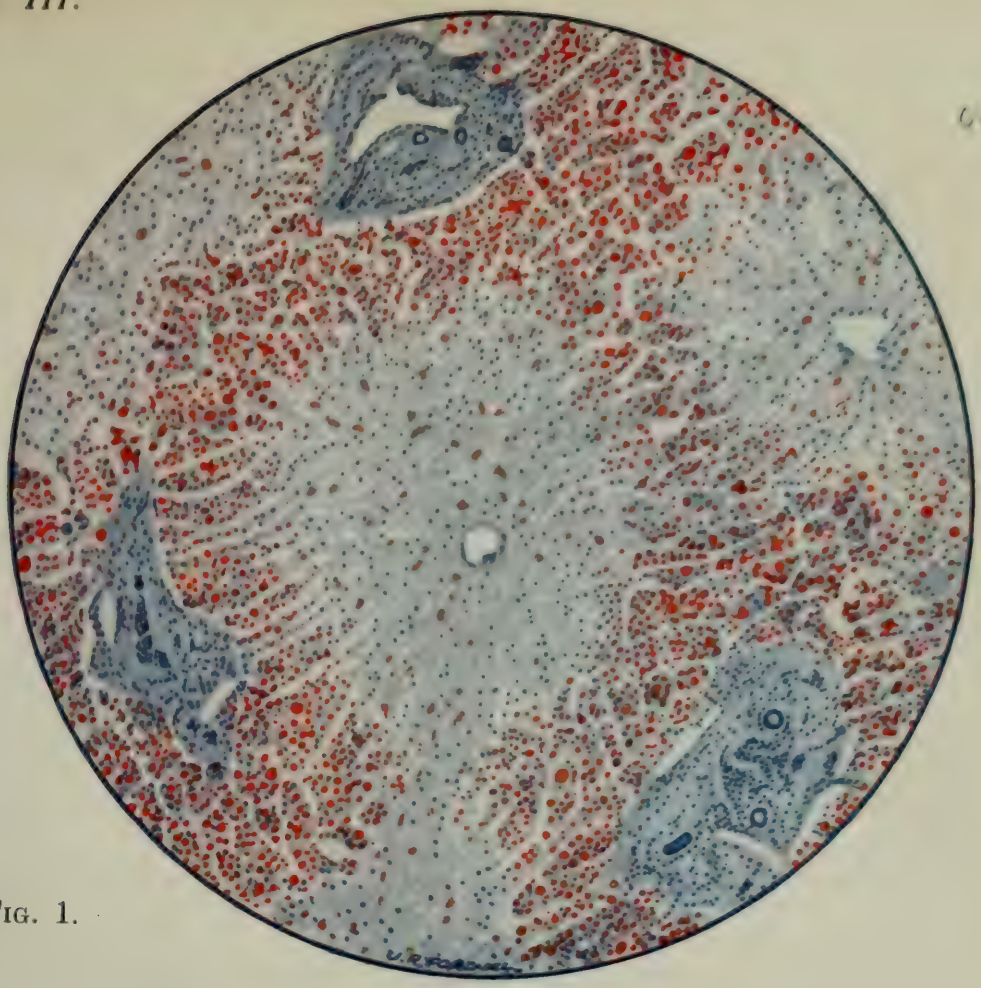


FIG. 1.

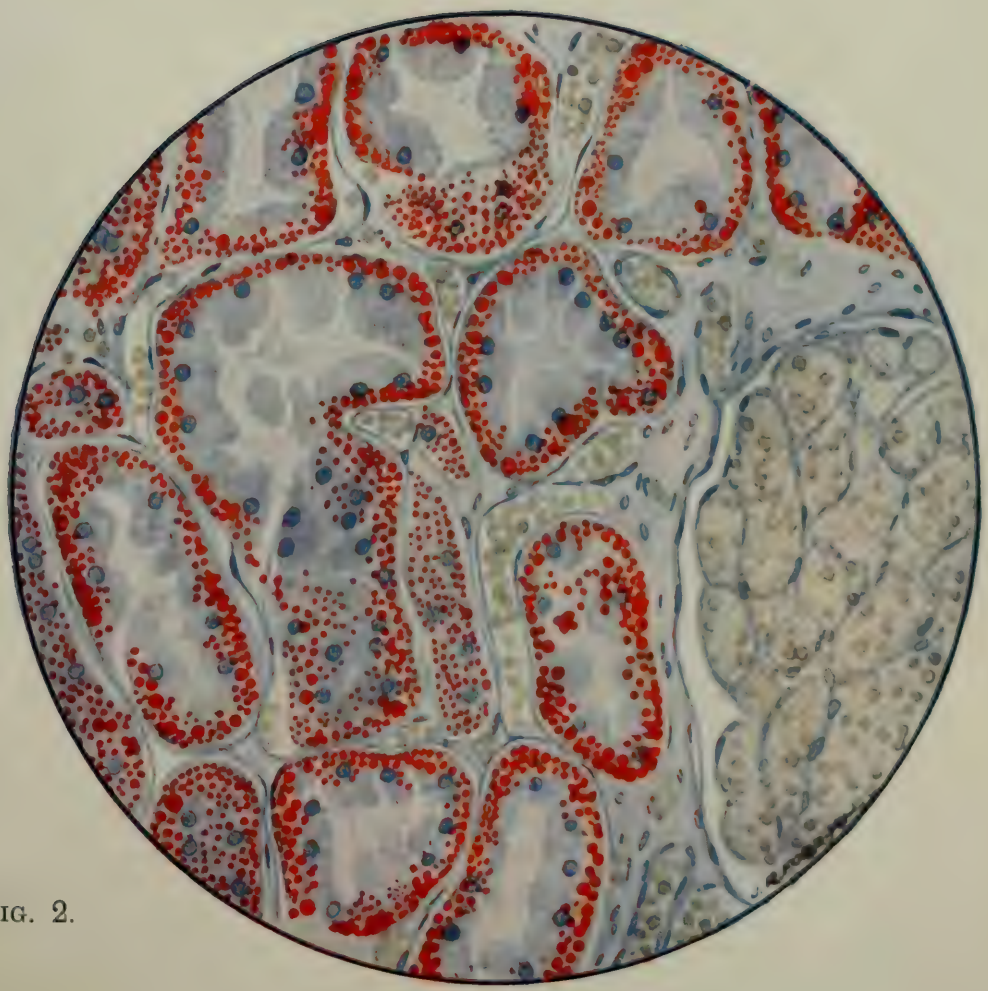


FIG. 2.

PLATE IV

FIGURE 1

Liver. Case III. Weigert's iron-haematoxylin and van Geison's mixture. (× 136.)

Includes part of one portal system, and bile ductules proliferating from three other portal systems in the periphery of a lobule. Fibres which were stained red are black. Original parenchyma replaced entirely by small, rounded, young, hepatic cells.

FIGURE 2

Liver. Case III. Haematoxylin and eosine. (× 230.)

Includes a portion of a portal system and of an hepatic lobule. Eosinophil leucocytes stippled black. Demonstrates: spongy, fatty remnants of original hepatic columns; young hepatic cells, formed by regenerative proliferation; proliferating biliary ductule; inflammatory infiltration with lymphocytes, endothelial cells and leucocytes.

PLATE IV

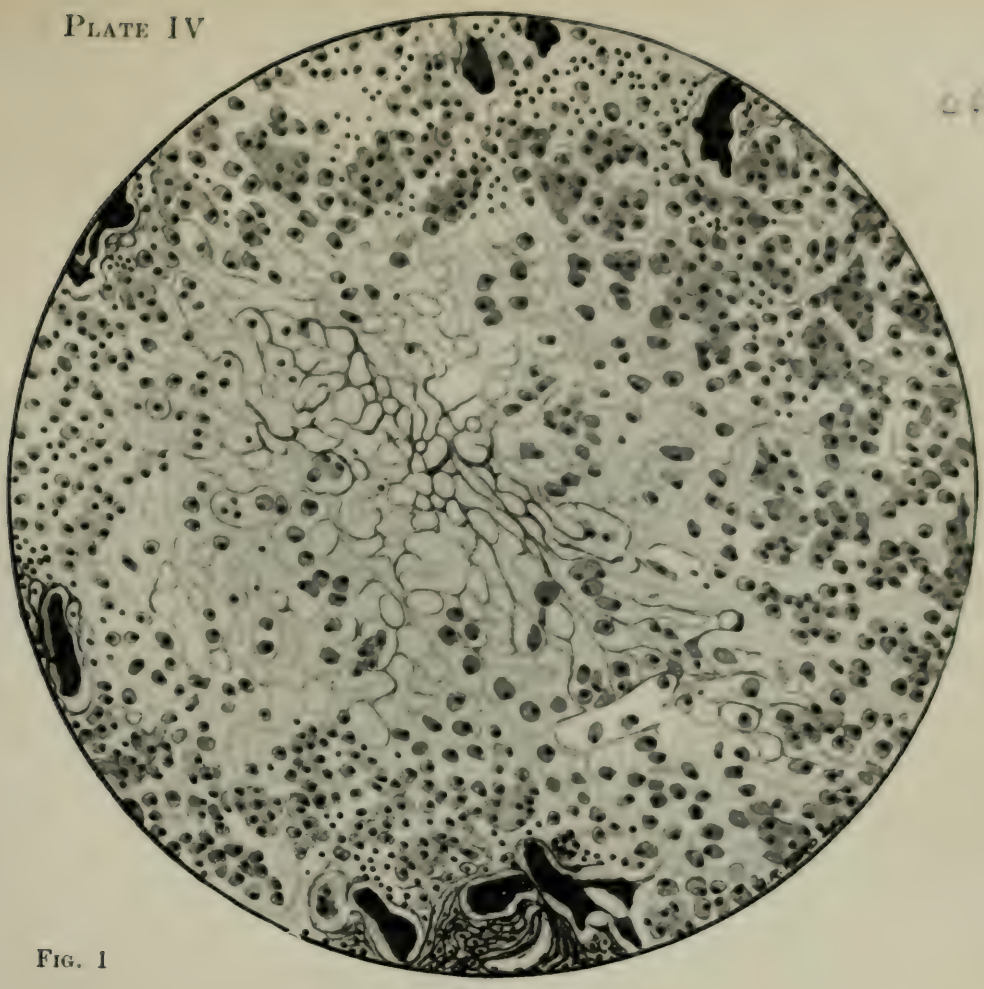


FIG. 1



FIG. 2

PLATE V

FIGURE 1

Liver. Case VIII. Haematoxylin and eosine. (× 185.)

Includes central vein and one portal system. Demonstrates: very extensive destruction of lobule; engorgement and haemorrhage in centre of lobule; young, regenerated, hepatic cells in periphery; proliferating biliary ductules.

FIGURE 2

Liver. Case VIII. Haematoxylin and eosine. (× 185.)

Includes a central vein and portions of two portal systems. Demonstrates: less extensive destruction of lobule, a rounded group of the original hepatic columns being present in the periphery. Among the cells of these columns, and in the remainder of the lobule, are young hepatic cells.

PLATE V

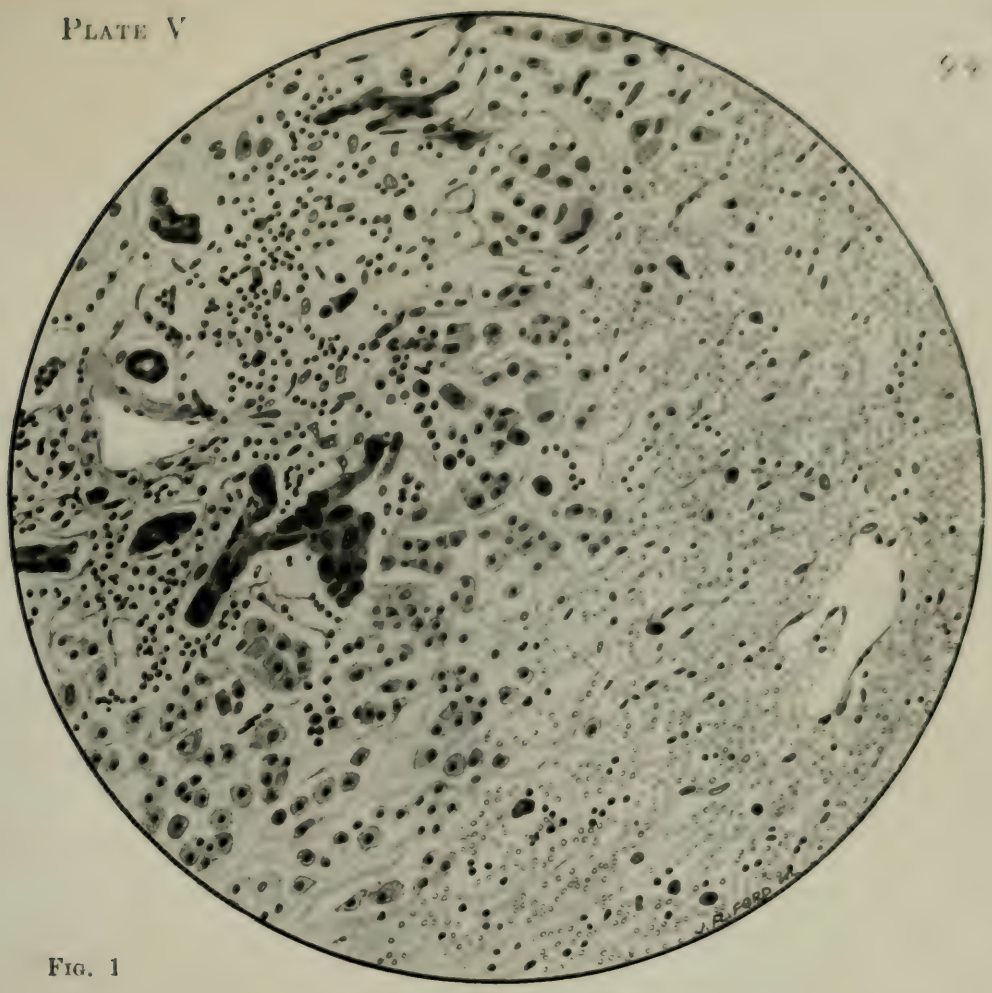


FIG. 1



FIG. 2

PLATE VI

FIGURE 1

Liver. Case of trinitrotoluene poisoning. Female, aged 15 years. Death ten days after appearance of jaundice and cessation of work. Haematoxylin and eosine. (× 23.)

Demonstrates area of complete destruction of hepatic lobules: 'red atrophy'.

FIGURE 2

Liver. Same case of trinitrotoluene poisoning. Haematoxylin and eosine. (× 23.)

Demonstrates area of partial destruction of hepatic lobules: early 'portal fibrosis'.

Plate VI.

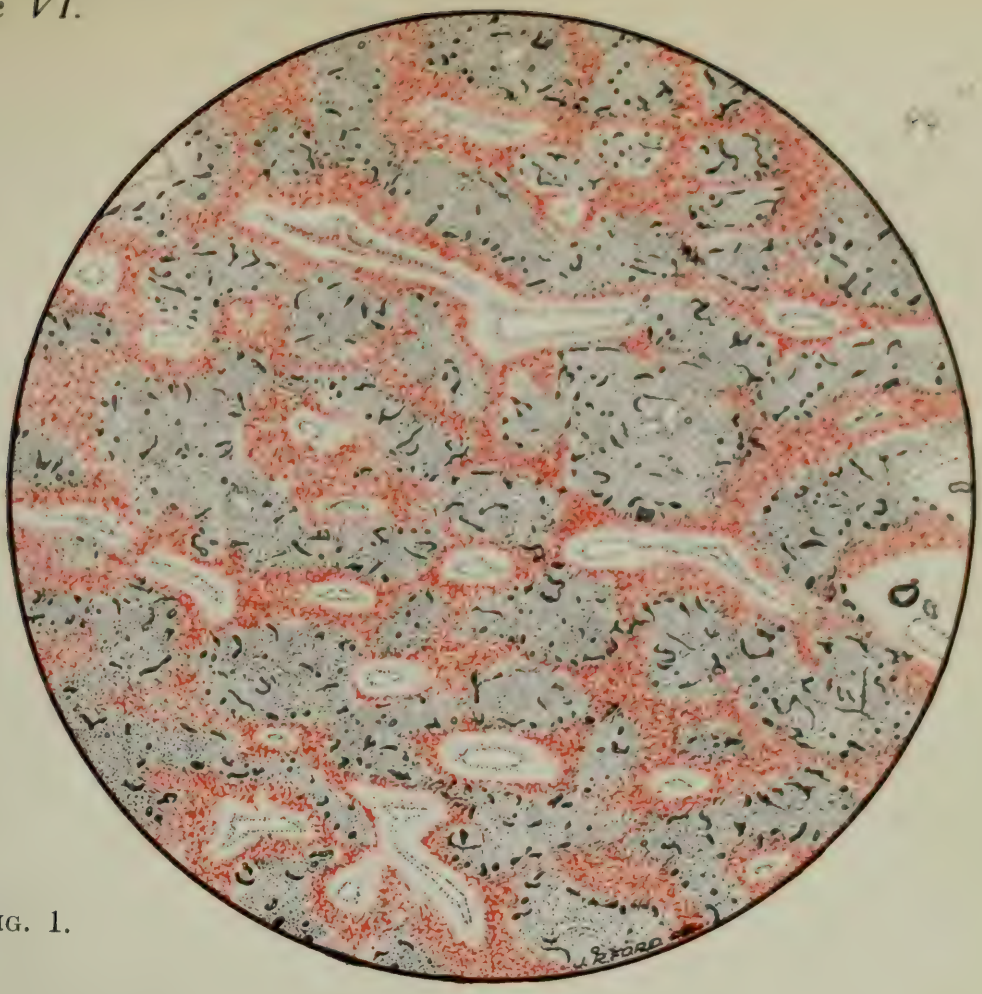


FIG. 1.

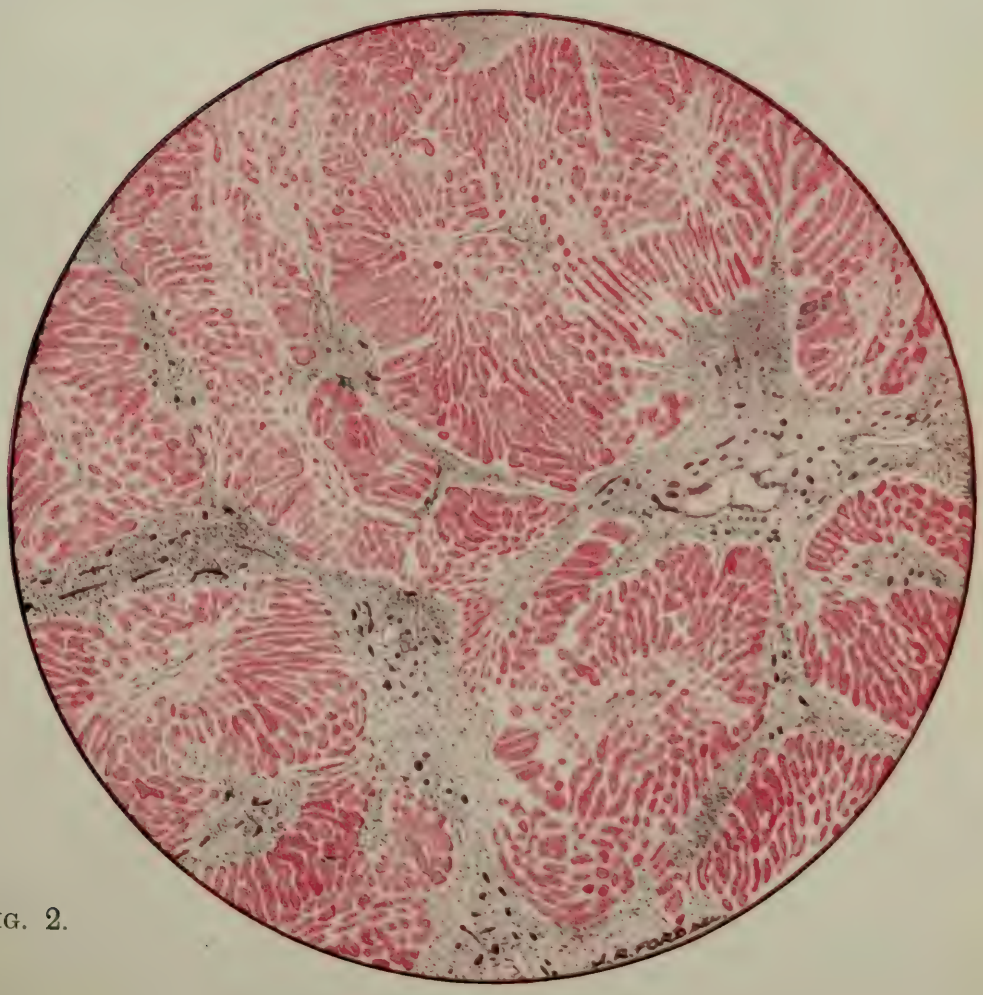


FIG. 2.

II. THE INFLUENCE OF SALVARSAN TREATMENT ON THE DEVELOPMENT AND PERSISTENCE OF IMMUNITY, AS INDICATED BY MEASUREMENTS OF AGGLUTININS

BY

E. W. AINLEY WALKER, D.M., D.Sc.

THIS investigation formed part of an inquiry undertaken in collaboration with Professor Gunn, the object of which was to determine, so far as possible, what influence, if any, the administration of salvarsan in animals exerts on their resistance to a bacterial infection and the action of toxins.

At the outset it appeared that a careful study of the immunity reactions in animals placed under salvarsan treatment was likely to afford valuable information as to the nature of any changes in the normal response to infection which might be brought about by the administration of the drug. And in the first instance the reaction of agglutinin production was selected for detailed examination, since agglutinins are the most easily and the most accurately measurable products of immunization. (Professor Gunn's experiments dealt with the question of toxins.)

Immunization.

Rabbits were used. In general they were immunized by single small intravenous injections of Standard Cultures (dead bacilli) of one or other of the enteric group: *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*. In many animals a second organism of the group was inoculated at a later stage, so that two independent agglutinin curves in different phases were available for study.

The dosage employed was always 0·1 c.c. of Standard Culture (diluted with normal saline solution to 1 c.c.) containing approximately 25,000,000 dead bacilli. But in the case of No. 1 three equal immunizing inoculations of 0·1 c.c. were made on days 1, 8, and 16. And in three cases (Nos. 6, 9, 10) the bacillus originally used for inoculation was again introduced in *twice* the original dose after the lapse of nearly twelve weeks of observation and salvarsan treatment.

All inoculations were made in the morning after the day's sample of blood had been withdrawn, and the day of inoculation was counted as day 1 of the experiment.

Salvarsan Dosage.

Two forms of the drug have been used for intravenous injection. The first was a sample of neosalvarsan (indicated in the tables as NS) obtained by Professor Dreyer and myself from the late Professor Ehrlich some years ago for experimental purposes: the second novarsenobillon (indicated as NAB).

The doses recorded in the tables are expressed as quantities per kilogramme of the body-weight of the animal at the date of the first salvarsan injection. Their relative values in animals of different weights depend on the actual concentration of the drug produced in the blood.[1] And this depends in turn on the blood-volume of the animal, which is proportional to its body-surface and not to its weight, and may be calculated from the formula $B = \frac{W^{0\cdot72}}{k}$ where the k for rabbit is 2·37.

Doses of 0·01, 0·02, and 0·03 grammes per kilo of body-weight were employed in the case of neosalvarsan, which was the sample first used, and doses of 0·03, 0·04, and 0·05 grammes per kilo of novarsenobillon. The dose most often used was 0·05 grammes per kilo of novarsenobillon. It was employed in all the later part of the work.

In Table I below are given the weights of the fifteen rabbits used for immunization at the time of the first injection of salvarsan, their theoretical blood-volumes read off from Dreyer and Ray's chart [2] and the percentage concentration of the drug in the blood calculated for a dose of 0·05 grammes per kilo of body-weight.

TABLE I.

Number of Animal.	*Sex.*	*Weight of Animal. Grammes.*	*Blood-volume of Animal. c.c.*	*Dosage 0·05 grammes per kilo.* *Actual dose. Grammes.*	*Dose per 100 c.c. of blood.*
1	m.	2,640	121	0·132	0·109
2	f.	1,900	97	0·095	0·098
3	m.	1,800	94	0·090	0·096
4	m.	1,820	94	0·091	0·097
5	m.	2,050	102	0·103	0·100
6	m.	2,600	120	0·130	0·108
7	f.	2,150	105	0·108	0·103
8	m.	2,140	105	0·107	0·102
9	m.	2,400	114	0·120	0·105
10	m.	2,080	103	0·104	0·101
11	m.	2,400	114	0·120	0·105
12	f.	2,700	123	0·135	0·110
13	m.	2,740	124	0·138	0·111
14	f.	2,180	106	0·109	0·103
15	f.	1,700	90	0·085	0·094
				Average	0·103

The concentration of the drug in the blood ranges from 0·094 to 0·111 per cent., a range of variation of about 16 per cent. The average of the concentrations is 0·103. To produce a similar concentration in the blood of a man of 70 kilos. possessing something

[1] Dreyer, G., and Walker, E. W. Ainley, *Proc. Roy. Soc. B.*, 1914, 87, 319.
[2] Dreyer, G., and Ray, W., *Phil. Trans. Roy. Soc.*, Series B, 1910, 201, 133.

over 5 litres of blood, a dosage of from 5 grammes to 5·5 grammes would be required.

But that this maximum dosage employed in rabbits did not approach a toxic dose was shown by the fact that twice this quantity per kilo (i. e. 0·1 grammes per kilo) produced no ill effects, nor any appreciable loss of weight, in the two animals on which it was tested. In one of these (weight 1,580 grammes, blood-volume 86 c.c.) the concentration of salvarsan in the blood was 0·184 per cent. In the other (weight 1,420 grammes, blood-volume 80 c.c.) the blood concentration of the drug was 0·178 per cent. Moreover, in the latter case the solution of novarsenobillon had been allowed to stand for six hours (two hours at 37° C. and four hours at the room temperature) before it was injected into the circulation.

The dates of inoculation and of salvarsan injection and the doses given are shown in the tables.

1 rabbit, No. 6, received altogether 9 doses of salvarsan.
1 rabbit, No. 9, received 5 doses of salvarsan.
3 rabbits, Nos. 3, 4, 10, received 4 doses of salvarsan.
2 rabbits, Nos. 2, 5, received 3 doses of salvarsan.
5 rabbits, Nos. 1, 7, 8, 12, 13, received 2 doses of salvarsan.
3 rabbits, Nos. 11, 14, 15, received 1 dose of salvarsan.

Time of Dosage.

In all cases the injections of salvarsan were given in the morning *after* the day's sample of blood had been withdrawn for examination. In different instances the drug was administered

(*a*) before an immunizing inoculation of bacterial culture,
(*b*) during the period of rapid rise in agglutinin production,
(*c*) during the period of sharp fall in the agglutinin curve,
(*d*) during the later period of (expected) gradual fall.

Thus one rabbit (No. 6) received four doses of salvarsan before its *first* immunizing inoculation.

Three rabbits (Nos. 6, 9, 10) received several doses of salvarsan before their second immunizing inoculation with a double dose of the bacterial culture previously employed.

Four rabbits (Nos. 1, 2, 7, 8) received one dose of salvarsan before immunization with a second bacterial culture.

Further, the first dosage with salvarsan following any particular immunizing inoculation was distributed as shown in Table II.

TABLE II.

First dose of salvarsan after immunizing inoculation given on day	*Rabbits Nos.*
2	4
4	3, 6, 8, 9
5	2, 7
6	3, 5
7	2
11	6, 7, 9, 10, 12, 13, 14, 15
26	1
28	10
39	1

Subsequently salvarsan treatment was repeated at intervals in many cases as recorded in the tables appended.

Health and General Condition of the Animals.

Of rabbits Nos. 1 to 12, whose treatment was begun in May and June 1918, all but one are still alive and in good condition at the time of writing in March 1919. Rabbit No. 9 died at the end of December 1918, four months after its last salvarsan injection. *Post mortem* it was found to be heavily infected with Coccidiosis. The treatment of rabbits, Nos. 13, 14, 15, was begun in September 1918. Nos. 13 and 14 remain alive and in good condition. No. 15, which received one injection of salvarsan in September, continued to grow and put on weight up to the end of October; but died with coccidial infection at the end of December.

Observations.

The effects of salvarsan treatment on the development and course of the agglutinin curve was followed by means of daily, or almost daily, observations of the titre of the serum for considerable periods, extending in a number of cases to 80 or 90 days, and in one instance to 108 days. Dreyer's method of measuring agglutinins was used. And in all cases the successive samples of serum were tested throughout each experiment on the *same batch* of Standard Culture as had been used for the immunizing inoculation. The readings of titre are expressed in agglutinin units (Dreyer).

Errors and Corrections.

Three sources of error require consideration, namely: (1) Experimental error in carrying out the agglutination tests; (2) effects due to the removal of the daily sample of blood; (3) the possibility that changes in blood-volume (concentration or dilution) are produced by the injection of salvarsan intravenously. As regards errors of technique, the conclusion is put forward on the results of numerous tests carried out in duplicate, and at times in triplicate, on particular serums that changes in titre recorded in these experiments exceeding 10 per cent. of rise or fall represent *real* changes in the agglutinin content of the serum, and fall outside the limits of probable experimental error.

The effect of the removal of a sample of blood every day, or almost every day, must be taken into account. Something like 1 to 1·5 per cent. (and perhaps sometimes 2 per cent.) of the blood is withdrawn when the sample is taken. The results are inappreciable during the period of rapid rise in the agglutinin curve. But they tend (as is well known) to produce some *irregularity* in the fall of the curve, not only by loss of agglutinins in the blood removed, but more especially by evoking a reaction of regeneration in the blood-forming organs. It is, however, evident that no *large* or sudden variations from the normal form of the agglutinin curve are likely to be attributable to this cause.

Certain drugs are known to produce temporary changes in blood-volume. It is therefore of importance to ascertain whether, and if so how far, apparent changes in the agglutinin content of the serum following an injection of salvarsan may be due to a concentration or to a dilution of the blood resulting from the introduction of the drug. Some experiments have been carried out along this line, but the problem has not yet been studied in detail. Yet it may be stated that the injection of a solution of salvarsan appears in general to cause some temporary *dilution* of the blood, which may amount to 10 or 12 per cent. in the first two or three days. This dilution will *per se* produce a fall in agglutinin titre, though *not* a fall equivalent to the fall in the concentration of the blood, since the tissue fluid taken up into the circulation itself possesses (as is known) a considerable content of agglutinins.

Hence, taking all the sources of error into due account, it would appear to be justifiable to conclude that an allowance of some 20 or 25 per cent. gives a very ample margin for all possible corrections. Changes in the normal form of the agglutinin curve of greater extent than this within the first few days after the injection of a dose of salvarsan; and changes on other occasions exceeding 10 per cent. may therefore be regarded as significant for the present purpose.

Results.

In discussing the results which have been obtained it is essential to refer briefly to the *form* and *time-relations* of the normal agglutinin curve which provides the sole standard of comparison.

The data of two such curves are included as controls in the tables below, and one of them is shown in Chart I. They show the slight irregularities due to experimental error and to the daily removal of a sample of blood. Points of importance are the following: In rabbits immunized with single intravenous inoculations of Standard Cultures of enteric group bacilli the marked rise in agglutinin titre begins to appear about the fourth day (the day of inoculation being counted as day 1, inoculations made in the morning, and the daily samples of blood withdrawn in the morning). The rise is rapid on the fifth, sixth, and seventh days. The maximum is usually reached, at any rate with the small inoculations used in these experiments, on the seventh to eighth day, though sometimes it is as late as the ninth day or as early as the sixth. Thus, taking the day of highest reading as the day of maximum (the true maximum necessarily lying on one side or other of the highest reading), I have records of its occurrence in sixteen cases, three times on day 6, six times on day 7, once on day 8, and five times on day 9, giving a mean of 7·7 days as the time of the maximum.

Thereafter a rapid fall sets in. The rate of fall diminishes day by day, and by the fourteenth to sixteenth day it has become relatively slow in comparison with the earlier rapid rate of diminution. Nevertheless it continues for some further period to be quite considerable in day to day readings, as is apparent when the figures

are plotted out on a fairly large scale. Subsequently the titre diminishes steadily, but with increasing slowness week by week; so that many months will pass before the immunization agglutinins cease to be readily measurable.

It will, of course, be fully recognized that at *every* stage of observation the only standards for comparison between animals treated with salvarsan and normal immunized animals are the general form and time-relations of the agglutinin curve. Its absolute height affords no criterion whatever, since individual idiosyncrasy in the animal plays so wide a rôle. Thus, for example the maximum titre of agglutinins found in different rabbits (or men

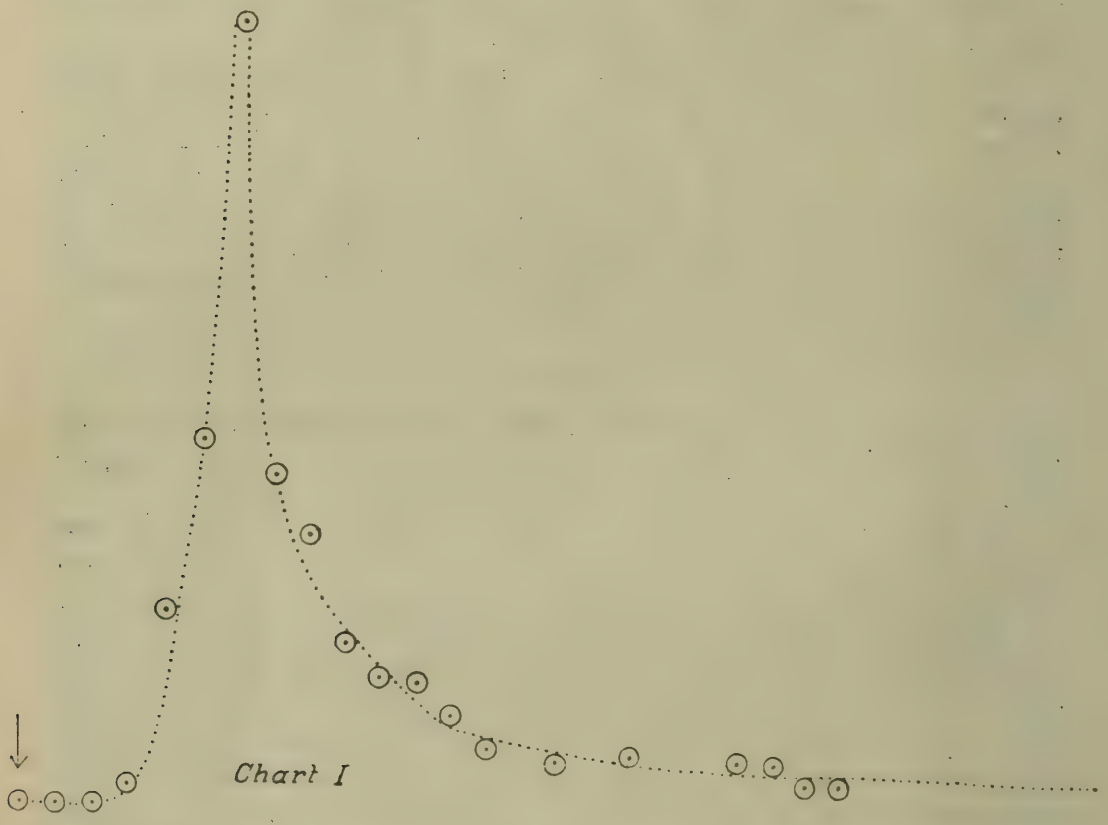


Chart I

inoculated with identically the same dose of antigen and by the same route, may differ by a *hundredfold or more*, as may also the different titres found on corresponding dates after inoculation. Another point to which attention may be drawn in passing is the frequency with which it is shown in the tables how inoculation with a second bacterium of the group causes a new rise in agglutinin titre for the bacterium used in the first immunization. The fact is, of course, familiar.

Bearing these points in mind, one may now proceed to examine the results obtained by treating rabbits with salvarsan before and during an immunity reaction.

1. The administration of four doses of salvarsan, extending over a period of twenty-four days and terminating three days before the first immunizing inoculation (total dose 0·06 grms. NS per kilo),

did not in No. 6 prevent a marked agglutinin response. Similarly, one dose of salvarsan before the immunizing inoculation with a second bacterium did not in Nos. 1, 2, 7, 8 prevent the appearance of a typical response.

2. Salvarsan (0·03 grms. NS per kilo) injected in No. 4 on the day after the immunizing inoculation did not appear to affect the data of maximum titre (day 7), or interfere with the development of a typical response.

In view of the results set forth in this and in the preceding paragraph it seems improbable that the introduction of a *therapeutic* dose of salvarsan would render the individual in any remarkable degree more susceptible than usual to intercurrent infection occurring at the same time, or quite shortly before or after the injection of the drug.

3. In rabbits treated with salvarsan during the period of rapid rise of agglutinin titre results were obtained which are set out in Table III.

TABLE III.

Salvarsan injection on day	*Dose per kilo of NS. Grammes.*	*Maximum titre occurred on day*
4	0·02	5 (No. 3)
4	0·03	6 (No. 6)
4	0·02	7 (No. 8)
4	0·03	6 (No. 9)
5	0·02	6 (No. 2)
5	0·02	6 (No. 7)
6	0·01	6 (No. 3
6	0·02	7 (No. 5)

Thus it appears that the *maximum* of the curve of agglutinin titre, which normally occurs as already stated on day 7 or 8, was usually ante-dated by the administration of salvarsan on day 4 or 5, or even on day 6. For in the eight animals above in Table III the maximum occurred in five on day 6, in one as early as day 5, and only twice as late as day 7. The mean of the observations is 6·1 as against the 7·7 for normal immunization—an ante-dating of the maximum by a day and a half.

4. This early maximum may be succeeded by the usual fall in titre, so that the whole curve up to the maximum and for a few days afterwards presents quite a normal form, though *ante-dated* in its phases, as already stated (cp. Nos. 2 and 5, Para. B agglutinins, Chart II).

But in other cases, instead of the sharp fall occurring at once, a plateau appears, and perhaps a later secondary rise in titre. Sometimes this kind of effect is so marked that the curve looks as if its top had been knocked off (cp. Nos. 3 and 7, Para. B agglutinins, Chart II). Or it may be that several points about the apex of the curve lie near together instead of being widely spaced as in normal curves (cp. Nos. 6 and 9, Para. A, and No. 8, Para. B agglutinins, Chart II).

In this chart, as also in Charts III, IV, and V, the different curves are drawn to different scales. The numbers beneath the curves are

the numbers of the rabbits under observation. Full data are given in the tables appended.

The conclusion suggested by the appearances just described is that the administration of salvarsan during the period of rapid rise in agglutinin production caused a temporary check in the reaction, followed by a stage of recovery and increased activity, which may manifest itself only in a greatly delayed rate of fall after the maximum, or may lead to a new rise in agglutinin titre. In other words, a kind of negative phase was produced which was succeeded by a phase of positive reaction.

5. For even in those cases in which the administration of salvarsan during the upstroke of the curve caused no obvious or

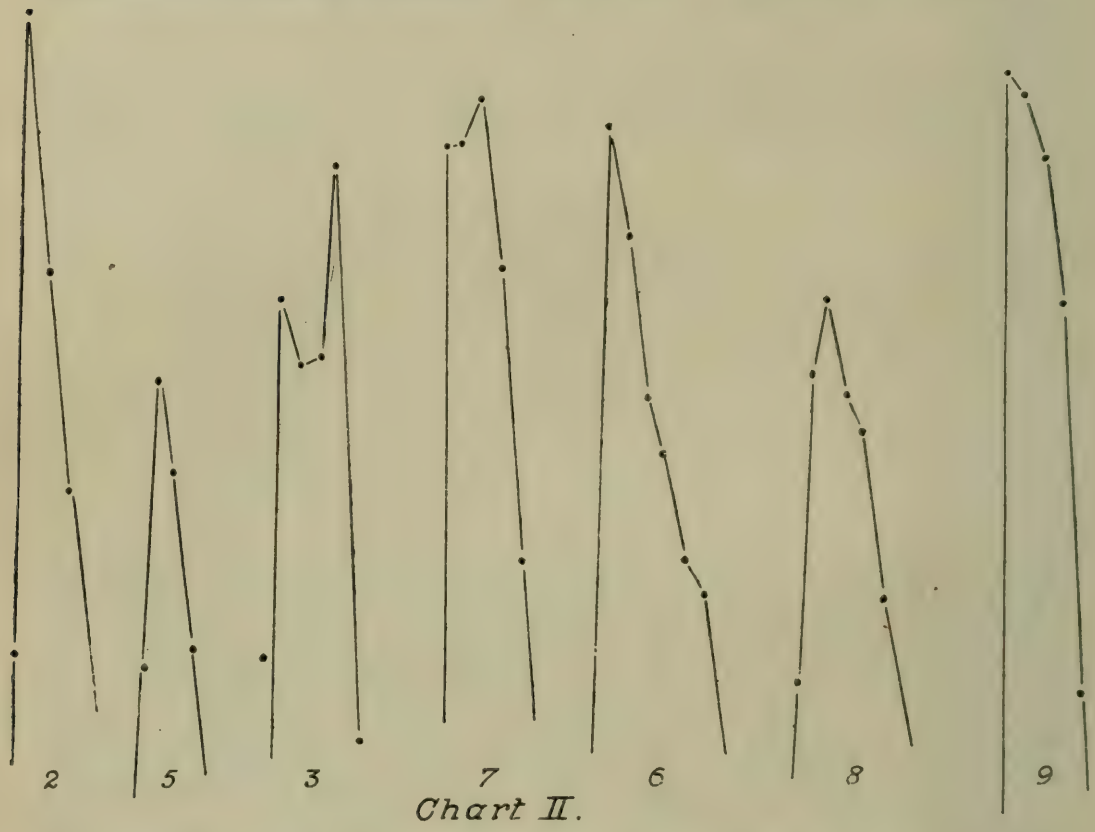


Chart II.

immediate change in its form (though usually ante-dating its maximum), as in Nos. 2 and 5 (Para. B agglutinins) already referred to—again in the case of No. 2 (Typhoid agglutinins), where salvarsan was injected on day 7, that is to say, about the time of probable maximum titre—and again in the numerous instances where it was injected on day 11, the data in the tables show that in *every* case evidence appeared within the next few days that the natural fall of titre was interrupted by the development of a positive phase of increased agglutinin titre.

The magnitude of this increase varies in different instances, so that it may appear (*a*) merely as a temporary check or succession of checks in the fall of titre, as in No. 9, days 10 and 11, days 12 and 13, and days 14 to 30, where it is seen that the titre is still *as high on the thirtieth day as it was on the fourteenth*; (*b*) it may be

represented by a new rise of titre—or an irregular succession of rises—amounting in different instances to such increases as the following above the point on the curve from which it starts:

46 per cent. in No. 3, Para. B agglutinins (days 26 to 28);
46 per cent. in No. 8, Typhoid agglutinins (days 65 to 66):
76 per cent. in No. 9 (days 94 to 95);
91 per cent. in No. 5 (days 12 to 14);
93 per cent. in No. 10 (days 93 to 97);
97 per cent. in No. 13 (days 11 to 12):



Chart III

128 per cent. in No. 6 (days 91 to 95);
215 per cent. in No. 14 (days 16 to 26);
288 per cent. in No. 15 (days 16 to 25).

Chart III is introduced to illustrate some of these rises in titre which occurred under salvarsan treatment at a time when otherwise a steady fall was to be expected.

6. On following out any particular table during the later stages of the agglutinin reaction it will be observed that in general each *repetition* of salvarsan injection gives rise to the appearance of negative and succeeding positive phases, or, as it may be expressed, a fall and recovery in the agglutinin titre. Sometimes one phase.

sometimes the other is the more marked; and in certain cases the negative phase did not appear at all as a fall in the day to day readings, while in other cases its appearance was delayed for a day or two.

The phase of 'recovery' may at times proceed no further than the mere restoration of titre to its former level, but more frequently it leads on to a considerable and perhaps prolonged new rise in agglutinins (see §§ 8 and 9 below). It is also noteworthy that while the downstroke of the agglutinin curve is normally one of regular and constantly diminishing curvature, the effect of salvarsan is in many cases to produce marked irregularity, so that a succession of falls and rises of considerable amplitude may follow one another.

7. On the whole it appears to be quite evident that when dosage with salvarsan is repeated at short intervals the effect of each succeeding dose upon agglutinin titre is less and less. The response diminishes; particularly as regards the development of the phase of rising titre. But in this, as in all other respects, different animals showed marked individuality and idiosyncrasy. In rabbit No. 13, for example, no obvious response was detected on administering the second dose of salvarsan, although the response to the first had been one of very striking amplitude.

8. It would be important to determine whether repeated dosage with salvarsan, in addition to exhausting the agglutinin response to the injection of the drug, has the possible effect of leading to a gradual exhaustion of the mechanism of reaction to infection. But since a particular animal can only be compared with *itself* as regards the absolute height of the maximum agglutinin titres obtained in response to successive immunizing inoculations with any given antigen, the method of approaching this question must be to compare the results obtained on the first occasion with those obtained in response to a second inoculation, when the animal has been subjected to salvarsan treatment in the interval between the two immunizing inoculations.

Here a further difficulty arises in the fact that, at any rate when equal doses of antigen are used, the response to a second inoculation may (as is known from the experiments of Jörgensen) be either greater, equal to, or less than the original response. Accordingly the fact that a less response was obtained to a second inoculation after salvarsan treatment than was obtained at the first inoculation would fail to afford evidence on the point at issue.

It seemed possible, however, that if a *double* dose of antigen was given on the second occasion, and that, despite this fact, the second response was invariably very much smaller than the first in a fairly long series of animals, a conclusion might be reached.

Owing to untoward events, only three such experiments have as yet been brought to completion. The results are referred to here for what they are worth, since so far as they go they are in mutual agreement. Rabbits Nos. 6, 9, 10 each had three doses of salvarsan between the maximum of the first response and the administration of a second (double) dose of the same antigen. In Nos. 6 and 9

there had also been an injection of salvarsan during the development of the first response.

In No. 6 the first maximum was 2650 units, the second only 392.
In No. 9 the first maximum was 550 units, the second only 324
In No. 10 the first maximum was 1460 units, the second only 440.

But while these results might seem to favour the view that the power of response had been partially exhausted in these animals, it is to be noted that in each case a subsequent injection of salvar-

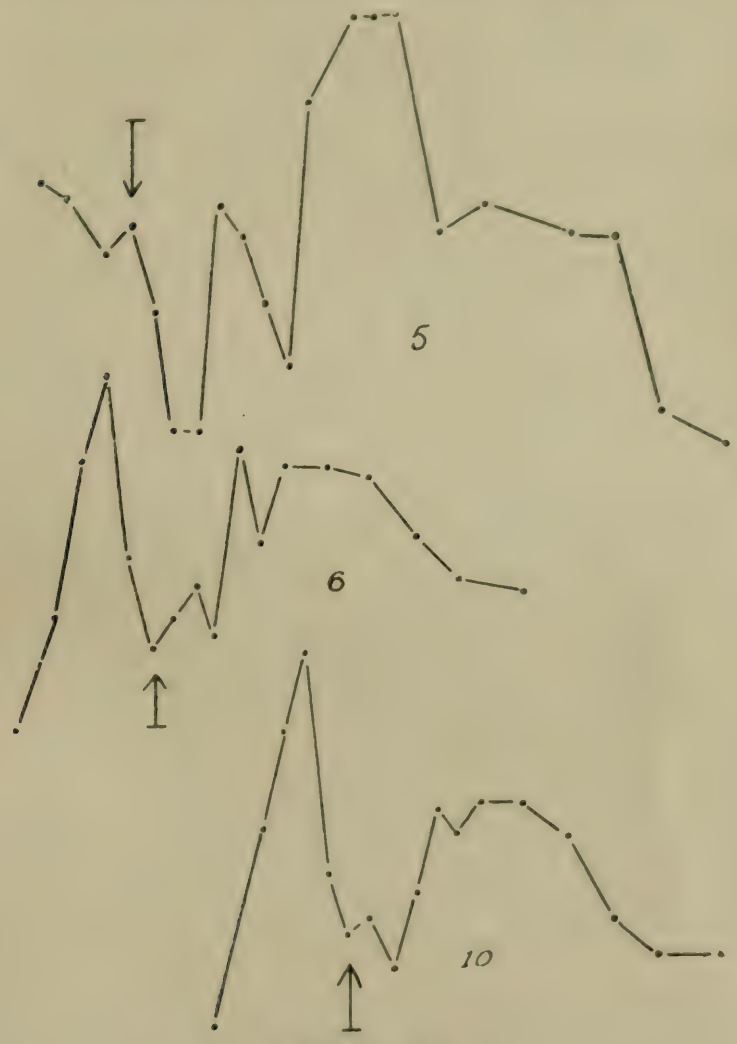


Chart IV.

san produced its usual effects in marked degree, the positive phase amounting to 128 per cent. in No. 6, 76 per cent. in No. 9, and 93 per cent. in No. 10. The question must therefore be regarded as still quite open.

9. In a proportion of cases the positive phase following the injection of salvarsan may become very *marked* indeed, and a great rise in titre may be produced at a time when otherwise a steady fall would be in progress. The rise may amount to 200 or 300 per cent. and even more. Thus in No. 3 (Typhoid agglutinins), following the injection of salvarsan on day 6, the agglutinin

titre maintains a level for two days and then rises in the next two days by 70 per cent. In the same table (Para. B agglutinins) the administration of salvarsan on the twentieth day of the Para. B curve (day 37) leads to an enormous rise in titre from 83 units to 383 units in the course of ten days, a rise of 360 per cent. In No. 5 salvarsan injection on day 23 gives an eventual rise in titre from the lowest point, 625 units, to 1100 units (76 per cent. rise). In No. 6 salvarsan on the ninety-first day of observation, ten days after an immunizing inoculation, gives a rise from 142 to 324 units

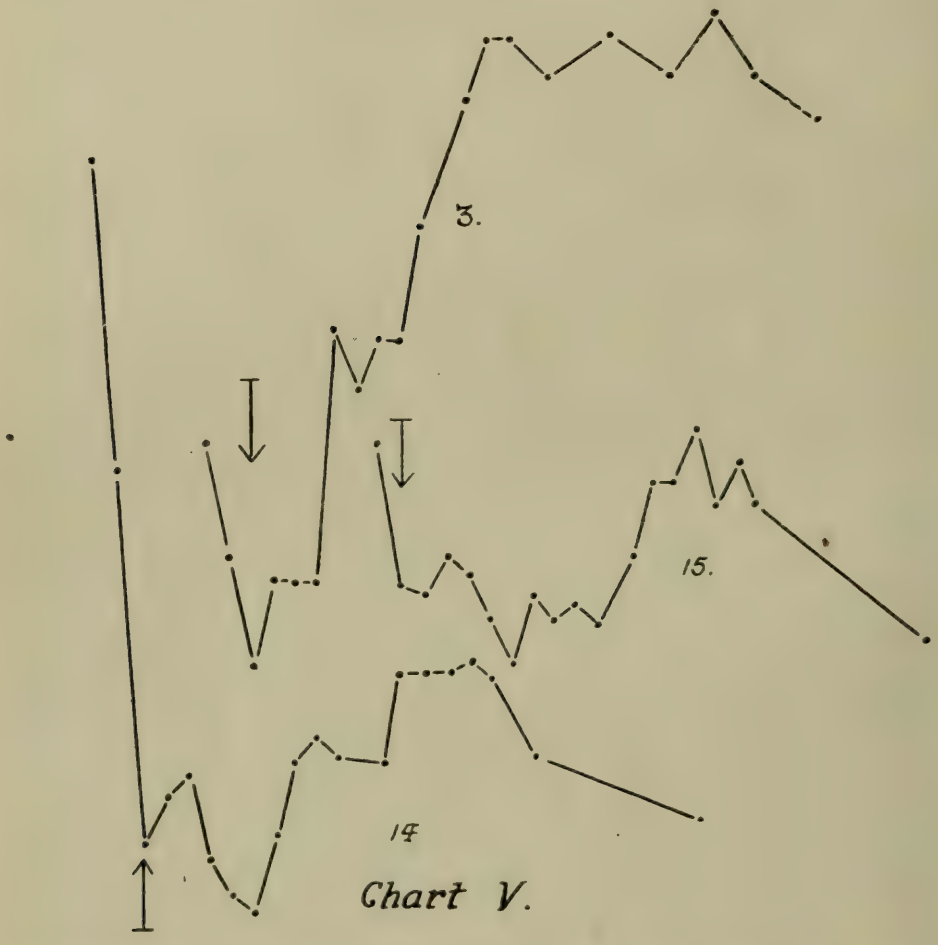


Chart V.

(128 per cent.); and in No. 10 (day 91 et seq.) the amplitude of the rise produced is from 156 units to 306 units (96 per cent.). Earlier in the table for No. 10 there are successive rises from 200 to 254 units (27 per cent.), from 156 to 280 units (79 per cent.), and from 176 to 272 units (54 per cent.). In No. 14 there is a rise from 55 units to 173 units (215 per cent.), and in No. 15 one from 8 units to 31 units (288 per cent.) following salvarsan injections (see Charts IV and V).

10. Of equal or even greater interest are the cases in which salvarsan treatment appears to lead not merely to a rise in agglutinin titre, but to a continued *maintenance of the level* thus reached. In this respect the Typhoid agglutinins of No. 3 are remarkable. As is seen in the table, the response to the typhoid inoculation is

poor, the titre reaching only 28 units on the sixth day, when the first injection of salvarsan was given. A further rise then follows after an interval of two days. The subsequent course of the curve and the treatment given are recorded in the table, from which it appears that the titre *remained raised* for 84 days to the end of the period of observation, and was then still 35 per cent. higher than at the date of the first salvarsan injection. A further point to be noted in the same table is the gradual slow rise in titre from day 33 to day 59, amounting to 52 per cent. in the twenty-six days. At the same time the rise in Para. B agglutinins following a salvarsan injection on day 37 is maintained from day 47 to day 61.

A similar prolonged plateau is seen in the typhoid agglutinins in No. 4 from about day 16 to day 57, and to a less extent in the later part of the curve of Para. B agglutinins (day 37 to day 82). Effects of the same kind are seen in Nos. 14 and 15. Here, then, we have maintenance of titre for a period varying from three to twelve weeks at a time when normally the titre should diminish week by week.

11. In fact the production and maintenance of this striking rise in titre of agglutinins constitutes in a number of cases *the main and outstanding* effect of the administration of salvarsan. It is, therefore, not improbable that a similar effect of salvarsan treatment may occur in relation to other serological reactions. Hence it would follow that in the case of reactions less sensitive and tests less delicate than are afforded by agglutinins, the *enhancement* consequent upon the injection of salvarsan might again bring into view a reaction which had previously fallen below the limit of recognition by existing methods. In view of this consideration the facts observed have an unexpected bearing on the phenomenon termed a 'provocative' Wassermann reaction. They suggest that the Wassermann reaction, which (as is well known) may again become positive in man after salvarsan treatment, although it had previously disappeared, is in reality dependent on a true immunity reaction, whose enhancement after the injection of salvarsan is in many ways similar to the enhancement of agglutinin production here described.

12. From their bearing on questions of general scientific interest the records of No. 12 are of importance. This rabbit, which had its maximum for Typhoid agglutinins on day 8, showed a marked fall in titre on day 9 and fell again on day 10. But the titre began to rise a little on day 11 even *before* the administration of salvarsan on that date. Thereafter there was a great rise in titre on days 12 and 13 to nearly 50 per cent. above the previous maximum, and the titre remained at a very high level for 14 days. A fall then set in. It transpired that this animal had become pregnant during the course of the experiment, and on day 34 it aborted six young. These were near full time in size and appearance; they were alive at birth but died in a few hours. Now full time on day 34 would date the conception back to about day 7. On the other hand, the doe had not begun to prepare a nest by the day of abortion, and

this is usually done some days before full time. Probably conception occurred between days 9 and 13. It is therefore impossible without further data to determine clearly how far the remarkable course of the agglutinin curve was due to salvarsan injection and how far it was associated with the pregnancy. But the observations form an interesting record (Chart VI). It is interesting to



Chart VI.

note further that the blood of the mother on the day of abortion had identically the same agglutinin titre as pooled blood from the young. The two series of tubes were quite indistinguishable.

Conclusions.

The administration of salvarsan in rabbits does not prevent response to infection of the normal type, as measured by agglutinin production.

On the contrary, it commonly appears to induce fresh, and at times prolonged, activity in the agglutinin-forming mechanism at a time when the normal response would otherwise be on the wane.

But the first effect of the injection of salvarsan is to cause the appearance of a negative phase of agglutinin production. This phase is of moderate degree and is of comparatively brief duration.

The evidence obtained suggests, but fails to answer, the question whether the repeated administration of salvarsan may not tend eventually to diminish temporarily the power of response to the introduction of particular antigens.

The enhancement of agglutinin formation which takes place under salvarsan treatment throws an indirect light on the origin of the so-called 'provocative' Wassermann reaction.

Rabbit No. 1.

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Typhoid Agglutinins Units.*	*Para B Agglutinins Units.*
	Typhosus			
...	$\frac{1}{10}$ c.c.	...	...	...
	days 1, 8, and 16			
54	...	...	51	...
60	...	...	45	...
63	...	...	51	...
69	...	...	45	...
75	...	0·01	40	...
		NS		
76	...	...	42	...
77	...	...	33	...
78	...	...	23	...
79	...	...	18	...
81	Para B	...	46	0·7
	$\frac{1}{10}$ c.c.			
82	...	...	78	0·6
83	...	...	74	0·7
84	...	...	58	141
85	...	...	43	1,378
86	...	...	41	2,585
87	...	...	46	5,512
88	...	...	46	2,346
89	...	...	37	1,902
90	...	...	41	1,146
91	...	...	41	902
92	...	...	40	795
93	...	...	...	634
96	...	...	...	415
98	...	...	...	366
100	...	...	33	366
103	...	...	29	305
104		...	28	298
105	...	...	26	225
106	...	0·05	24	230
		NAB		
107	...	...	22	146
108	...	...	19	98
109	...	...	19	102
110	...	...	16	107
111	...	...	22	138
112	...	...	22	122
113	...	...	21	115
114	...	...	22	122
116	...	...	21	122
117	...	...	22	112
118	...	...	23	107
120	...	...	15	83
123	...	...	13	80
126	...	...	13	61
128	...	...	13	61
131	...	...	12	61
134	...	...	13	61

Rabbit No. 2.

1	Typhosus	...	<0·5	...
	$\frac{1}{10}$ c.c.			
7	...	0·01	1,048	...
		NS		
8	...	...	933	...
9	...	...	546	...
10	...	...	501	...
11	...	...	396	...
13	...	...	237	...
16	...	...	422	0·6
17	Para B	...	291	0·7
	$\frac{1}{10}$ c.c.			
18	...	...	224	17
19	...	...	200	27
20	...	...	224	215
21	...	0·02	254	1,690
		NS		
22	...	...	254	3,476
23	...	...	197	2,756
24	...	...	172	2,146
25	...	...	131	2,146
26	...	...	116	1,220
27	...	...	103	868
28	...	...	67	654
30	...	...	179	610
32	...	...	164	476
35	...	...	149	305
36	...	...	149	295
37	...	...	127	254
38	...	0·05	127	246
		NAB		
39	...	...	119	254
40	...	...	85	220
41	...	...	85	195
42	...	...	84	185
43	...	...	61	146
44	...	...	54	140
45	...	...	52	122
46		...	66	220
48	...	...	75	215
49	...	...	90	215
50	...	...	84	220
52	...	...	60	146
55	...	...	60	144
58	...	...	69	168
60	...	...	66	151
62	...	...	46	129
65	...	...	54	146

Rabbit No. 3.

Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Typhoid Agglutinins Units.	Para B Agglutinins Units.	Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Typhoid Agglutinins Units.	Para B Agglutinins Units.
1	Typhosus	...	...	...	40	...	...	47	122
	$\frac{1}{10}$ c.c.				41	...	...	48	244
6	...	0·01	28	...	42	...	...	47	215
		NS			43	...	..	52	237
7	...	...	28	...	44	...	...	52	237
8	...	...	28	..	45	...	...	50	293
9	...	...	43	...	47	...	...	50	354
10	...	...	47	...	48	...	...	58	383
12	...	...	45	...	49	...	...	57	383
14	...	...	47	...	51	...	...	58	366
16	...	...	40	0·7	54	...	...	55	385
17	Para B	...	41	0·7	57	...	...	65	366
	$\frac{1}{10}$ c.c.				59	...	...	73	395
19	...	...	41	1	61	...	...	50	366
20	...	0·02	47	51	64	...	0·05	48	341
		NS					NAB		
21	...	...	45	537	65	...	...	47	244
22	...	...	28	500	66	...	...	47	317
23	...	...	28	502	67	...	...	27	341
24	...	...	25	610	68	..	...	47	341
25	...	...	...	293	69	...	...	50	341
26	...	...	...	205	70	...	...	52	261
28	...	...	...	295	71	...	...	52	293
30	...	...	...	259	72	...	...	52	268
33	...	...	48	190	73	...	...	54	244
35	...	...	48	190	75	...	...	60	244
36	...	...	48	134	76	...	...	52	244
37	...	0·05	47	83	77	...	.	47	219
		NAB			78	...	...	47	249
38	...	...	47	122	79	...	...	42	215
39	...	...	47	122	84	...	...	38	190

Rabbit No. 4.

1	Typhosus	...	...	...	37	...	...	200	250
	$\frac{1}{10}$ c.c.				38	...	...	196	235
4	...	...	19	...	39	...	...	200	250
5	...	...	126	...	40	...	...	200	250
6	...	...	214	...	41	...	...	196	220
7	...	...	243	...	42	...	...	196	250
8	...	...	243	...	43	...	...	194	250
10	Para B	...	214	1	45	...	...	214	250
	$\frac{1}{10}$ c.c.				46	...	...	226	250
11	...	0·02	243	0·9	47	...	...	214	323
		NS			49	...	...	196	220
12	...		371	2	52	...	...	194	220
13	...	...	264	283	55	...	...	184	225
14	...	..	243	2,100	57	...	...	186	220
15	...	...	179	2,825	59	...	...	161	195
16	...	...	193	3,450	62	...	0·05	161	210
17	...	...	209	2,825			NAB		
18	...	0·02	183	2,200	63	...	...	126	235
		NS			64	...	...	111	225
19	...	...	196	1,700	65	..	...	97	220
20	...	...	206	1,950	66	...	...	86	213
21	...	...	209	1,250	67	...	...	111	220
22	...	...	206	1,050	68	...	...	106	213
23	..	...	..	708	69	...	...	111	218
24	...	...	...	638	70	...	...	111	218
26	...	...	...	550	71	...	...	111	218
28	..	...	...	535	73	...	...	143	218
31	...	...	206	438	74	...	...	111	250
33	...	...	214	395	75	...	...	126	263
34	...	...	206	...	76	...	...	126	263
35	...	0·05	194	354	80	...	...	94	250
		NAB			82	...	...	91	220
36	...	...	231	374					

Rabbit No. 5.

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para B Agglutinins Units.*
1	Para B $\frac{1}{10}$ c.c.	...	<0.6
4	...	...	17
5	...	...	263
6	...	0.02 NS	1,413
7	..	...	2,200
8	...	...	1,950
9	...	...	1,450
10	...	...	1,413
11	...	...	1,050
12	...	...	575
13	..	...	875
14	...	...	1,100
15	..	...	890
16	...	...	890
17	...	...	865
19	...	...	903
20	...	...	890
22	...	...	828
23	..	0·05 NAB	865
24	...	...	765
25	...	...	625
26	..	...	625
27	...	...	890
28	...	...	875
29	...	...	780
30	...	...	706
31	...	...	1,000
33	...	...	1,100
34	...	...	1,100
35	...	...	1,100
37	...	...	865
39	...	...	890
43	...	...	865
45	...	...	865
47	...	...	663
50	...	0·05 NAB	625
51	.	...	525
52	...	...	800
53	...	...	625
54	...	...	625
55	...	..	550
56	...	...	525
57	...	...	550
58	...	...	538
59	...	...	355
61	...	...	340
62	...	...	375
63	...	...	325
64	...	...	250
68	...	...	265
70	...	...	250

Rabbit No. 11.

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Typhoid Agglutinins Units.*
1	Typhosus $\frac{1}{10}$ c.c.	...	...
5	...	...	31
7	...	...	143
8	...	...	113
9	...	...	94
10	. .	...	89
11	...	0·05 NAB	89
12	...	...	86
13	...	...	80
14	...	...	113
15	...	...	63
16	...	...	76
17	...	...	43
18	...	...	63
19	...	...	57
21	...	...	50
22	...	...	56
23	...	...	54
25	...	...	63
28	...	...	43
30	..	...	36
32	...	...	33
35	...	...	34
92	...	...	14

Rabbit No. 6.

Received 0·01 grammes per kilo Salvarsan NS 24 days before day 1. .
,, 0·01 ,, ,, ,, ,, 17 ,, ,,
,, 0·02 ,, ,, ,, ,, 10 ,, ,,
,, 0·02 ,, ,, ,, ,, 3 ,, ,,

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para A Agglutinins Units.*	*Day of Experiment.*	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para A Agglutinins Units.*
1	Para A $\frac{1}{10}$ c.c.	...	4	42	...	...	200
				43	...		258
3	...	...	5	44	...	...	156
4	...	0·03 NS	45	45	...	...	156
				46	...	...	154
5	...	...	712	47	...	...	152
6	...	..	2,650	48	...		168
7	...	...	2,340	49	...	...	168
8	...	...	1,880	50	...	...	176
9	...	..	1,760	52	...	...	176
10	...	...	1,346	53	...	...	174
11	...	...	1,246	54	...	...	174
12	...	...	848	56	...	...	142
13	...	...	848	60	...	...	116
14	...	...	760	81	Para A $\frac{1}{5}$ c.c.	...	60
15	...	...	860				
20	...	...	560	82	...	...	57
24	...	...	500	84	...	...	57
27	...	...	440	85	...	...	77
28	...	0·03 NAB	390	87	...	...	176
				88	...	...	314
29	...	...	390	89	...	...	392
30	...	...	390	90	...	...	226
31	...		200	91	...	0·05 NAB	142
32	...	.	390				
33	...	...	390	92	...	...	176
34	...	..	370	93	...	...	200
35	...	0·04 NAB	320	94	...	...	156
				95	...	...	324
36	...		200	96	...	...	240
37	..	.	200	97	...	...	314
38	...	.	226	99	...	...	314
39	...	...	200	101	...	...	300
40	...	...	200	103	...	...	250
41	...	0·05 NAB	188	105	...	...	210
				108	...	...	200

Rabbit No. 7.

Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Typhoid Agglutinins Units.	Para B Agglutinins Units.
1	Para B $\frac{1}{10}$ c.c.	...	...	0·8
2	...	...	...	0·3
3	...	...	...	1·5
4	...	...	...	53
5	...	0·02 NS	...	583
6	...	..	...	2,075
7	...	..	...	2,075
8	...	...	...	2,158
9	...	...	...	1,845
10	..	...	...	1,300
11	..	...	...	850
12	...	..	...	775
14		...	...	688
16	...	...	...	488
19		...	...	350
46	...	...	...	125
47	...	...	...	120
48	...	...	...	110
49	...	...	...	110
54	Typhosus $\frac{1}{10}$ c.c.	...	...	...
58	...	...	486	250
60	...	...	914	250
61	...	...	629	220
62	...	...	486	141
63	...	...	429	125
64	...	0·05 NAB	404	125
65	...	...	500	110
66	...	...	414	90
67	...	...	414	185
68	...	...	420	110
69	..	...	420	110
70	...	...	379	65
71	...	...	336	75
72	...	...	329	65
73	...	...	286	...
74	...	...	271	55
75	...	...	257	...
76	...	...	229	49
78	.	...	214	...
81	...	...	193	...
83	...	...	200	...
85	...	...	186	25
88	...	...	200	...

Rabbit No. 13.

Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Para B Agglutinins Units.
1	Para B $\frac{1}{10}$ c.c.	...	.
4	...	...	3·75
5	...	...	4·25
6	...	...	12·5
7	...	...	25
8	...	...	49
9	...	...	75
10	...	...	78
11	...	0·05 NAB	38
12	...	...	75
13	...	...	60
14	...	...	45
15	...	...	30
16	...	...	27
17	...	...	36
18	...	...	31
19	...	...	25
20	...	...	24
22	...	0·05 NAB	21
23	...	..	21
24	...	...	19
25	...	...	16
26	...	...	13
27	...	...	10
29	...	...	8
37	...	...	4

Rabbit No. 8.

Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Typhoid Agglutinins Units.	Para B Agglutinins Units.
1	Para B $\frac{1}{10}$ c.c.	...	...	...
2	...	...	...	<0·6
3	...	...	...	0·8
4	...	0·02 NS	...	37
5	...	...	...	618
6	..	...	...	1,458
7	...	..	...	1,658
8	...	...	...	1,400
9	...	...	...	1,300
10	...	..	...	828
11	...	...	...	728
12	...	...	..	628
14	...	...	...	463
17	...	...	...	375
45	...	...	..	78
46	...	.	...	63
47	...		...	56
48	...	...	..	56
53	Typhosus $\frac{1}{10}$ c.c.	...	...	...
57	..	...	200	75

Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Typhoid Agglutinins Units.	Para B Agglutinins Units.
59	...	...	509	85
60	...	...	403	63
61	...	...	336	49
62	...	...	264	49
63	...	0·05 NAB	200	41
64	...	...	186	39
65	...	...	143	41
66	...	...	209	50
67	...	...	90	55
68	...	...	91	55
69	...	...	81	45
70	...	..	70	55
71	...	...	71	60
73	...	...	83	78
74	...	...	71	...
75	...	...	43	63
77	...	...	56	...
80	...	...	76	...
82	...	...	63	...
84	...	...	71	63
87	...	..	71	..

Rabbit No. 14.

Day of Experiment.	Inoculations.	Salvarsan dosage grammes per kilo.	Para B Agglutinins Units.
1	Para B $\frac{1}{10}$ c.c.		<0·8
4	...	...	6·5
5	...	...	20
6	...	...	89
7	...	.	148
8	.	.	275
9	...	..	368
10	.	...	220
11		0·05 NAB	89
12	.		110
13	...	..	120
14	.	...	80
15	..	..	63
16	..		55
17	...	..	90
18	..		125
19	...		135
20	.	...	128
22	..		125
23	..	...	168
24	...		168
25	...		168
26	...	...	173
27	...	..	135
29			125
37			98

Rabbit No. 9.

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para A Agglutinins Units.*
1	Para A $\frac{1}{10}$ c.c.	...	0·6
3	...	...	1·2
4	...	0·03 NS	12
5	...	...	110
6	...	...	550
7	...	...	540
8	...	...	510
9	...	...	440
10	...	...	264
11	...	...	260
12	...	...	196
13	...	...	196
14	...	...	88
15	...	...	102
20	...	...	78
24	...	...	88
27	...	...	86
28	...	0·03 NAB	86
29	...	...	86
30	...	...	86
31	...	...	68
32	...	...	88
33	...	...	58
34	...	0·04 NAB	74
35	...	...	88
36	...	...	60
37	...	...	58
38	...	...	66
39	...	...	62
40	...	...	76
41	...	0·05 NAB	50
42	...	...	58
43	...	...	76
44	...	...	39
45	...	...	40
46	...	...	40
47	...	...	44
48	...	...	50
49	...	...	39
50	...	...	42
52	...	...	47
53	...	...	42
54	...	...	42
56	...	...	42
60	...	...	37
81	Para A $\frac{1}{5}$ c.c.	...	26
82	...	. .	25
84	...	...	25
85	...	...	50
87	...	...	113
88	...	...	226
89	...	...	324
90	...	...	200
91	...	0·05 NAB	154
92	...	...	200
93	...	...	154
94	...	...	100
95	...	...	176
96	...	...	154
97	...	...	100
99	...	...	164
101	...	...	136
103	...	...	116
105	...	...	110
108	...	...	130
137	..	...	30

Rabbit No. 10.

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para A Agglutinins Units.*	*Day of Experiment.*	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para A Agglutinins Units.*
1	Para A $\frac{1}{10}$ c.c.	...	0·6	43	...	...	272
				44	...	...	176
3	...	...	1	45	...	...	132
4	...	...	12	46	...		138
5	...	...	174	47	..	...	142
6	...	...	1,166	48	...		142
7	...	...	1,460	49	...	...	142
8	...	...	1,020	50	...	...	142
9	...	...	880	52	...	...	138
10	...	...	662	53	...	...	100
11	...	...	520	54	...	...	100
12	...	...	406	56	...	...	132
13	...	...	300	60	...	...	100
14	...	...	280	81	Para A $\frac{1}{5}$ c.c.	..	58
15	...	...	250				
20	...	...	220	82	...	..	57
24	...	...	210	84	...	...	57
27	...	...	200	85	...	...	100
28	...	0·03 NAB	200	87	...	...	280
				88	...	...	370
29	...	...	226	89	...	...	440
30	...	...	254	90	...	...	240
31	...	...	200	91	...	0·05 NAB	188
32	...	...	200				
33	...	...	176	92	...	...	200
34	...	0·04 NAB	156	93	...	...	156
				94	...	...	226
35	...	...	240	95	...	...	300
36	...	...	226	96	...	...	280
37	...	...	200	97	...	...	306
38	...	...	240	99	...	...	306
39	...	...	280	101	..	...	280
40	...	...	260	103	...	...	200
41	...	0·05 NAB	176	105	...	...	176
				108	...	...	176
42	...	...	254	137	...	...	78

Rabbit No. 12.

Day of Experiment.	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Typhoid Agglutinins Units.*
1	Typhosus $\frac{1}{10}$ c.c.	...	...
5	...	...	61
7	...	...	429
8	...	...	714
9	...	...	457
10	...	...	429
11	...	0·05 NAB	471
12	...	...	943
13	...	..	1,019
14	...	...	943
15	...	...	843
16	...	...	914
17	...	...	857
18	...	...	857
19	...	...	843
21	...	...	857
22	...	...	871
23	...		857
25	...	...	886
28	...	...	714
30	...	...	429
32	...	...	371
35	...	...	357
37	...	...	279
39	...	...	243
40	...	...	271
42	...	...	314
44	...	...	207
45	...	...	207
46	...	0·05 NAB	229
47	...	...	184
48	...	...	196
49	...	...	203
50	...	...	143
51	...	...	151
52	...	...	193
54	...	...	200
56	...	...	193
58	...	...	193
60	...	...	162
63	...	...	193
92	...	...	161

Rabbit No. 15.

Day of Experiment	*Inoculations.*	*Salvarsan dosage grammes per kilo.*	*Para B Agglutinins Units.*
1	Para B $\frac{1}{10}$ c.c.	...	<0·8
4	...	...	3·25
5	...	...	5·5
6	...	...	13
7	..	...	24
8	...	...	38
9	...	...	58
10	...	...	30
11	...	0·05 NAB	16
12	..	...	15
13	...	...	19
14	...	...	17
15	...	...	13
16	...	...	8
17	...	...	15
18	...	...	13
19	...	...	14
20	...	...	12
22	...	...	19
23	...	...	26
24	...	...	26
25	...	...	31
26	...	...	24
27	...	...	28
29	...	...	24
37	...	...	10

Control A.

Day of Experiment.	*Inoculations.*	*Para B Agglutinins Units.*
1	Para B $\frac{1}{10}$ c.c.	0·7
2	...	0·6
3	...	0·7
4	...	141
5	...	1,378
6	...	2,585
7	...	5,512
8	...	2,346
9	...	1,902
10	...	1,146
11	...	902
12	...	795
13	...	634
15	...	415
17	...	366
19	...	366
22	...	305
23	...	298
24	...	225
25	...	230

Control B.

Day of Experiment.	*Inoculations.*	*Para A Agglutinins Units.*
1	Para A $\frac{1}{10}$ c.c.	0·6
3	...	1
4	.	12
5	...	174
6	...	1,166
7	..	1,460
8	.	1,020
9		880
10	.	662
11	..	520
12	.	406
13	..	300
14	.	280
15	.	250
20	.	220
24		210
27		200

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The Effects of Alcohol and some other Drugs during Normal and Fatigued Conditions



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INTRODUCTION

THE present report is the third of a series of memoirs published by the Medical Research Council at the request of the Central Control Board (Liquor Traffic). The earlier of the experiments now described were part of an investigation independently undertaken before the beginning of the war, but the later ones were devised to answer problems suggested to the authors by the Chairman of the Board.

In some respects the value of the experiments is enhanced, in other respects it is restricted by their at once extensive and limited character. They are extensive in so far as they deal with certain mental effects not only of alcohol, but of opium, strychnine, tea, chloroform, &c. But any loss of intensiveness arising from experiments over so wide a pharmacological field receives compensation from the similar and opposite actions revealed by their comparison. These drugs appear to fall into two antagonistic groups, (i) alcohol and chloroform, and (ii) strychnine, opium, and tea, in regard to the tests applied.

On the other hand, these experiments are limited in scope since they were carried out by only two subjects, the greater number indeed being performed on only one subject, who had never to her knowledge previously taken alcohol in any form. But although caution must be exercised in generalizing from conclusions thus based, they acquire more than ordinary value inasmuch as they issue from two experts well trained to avoid the inevitable pitfalls of human experiment. Thus they have followed Dr. Rivers's example of disguising the drugs taken and of employing control mixtures, indistinguishable from the disguised drugs and taken on the days when the latter were not taken, so that suggestion arising from foreknowledge could play no part in obscuring the true effect of the drug.

The writers' researches on the mental effects of alcohol will naturally receive principal attention. They find that, despite the subjective feelings of greater ease in carrying out the tests, alcohol produces in them a distinct loss of precision in the dotting test, a well-marked loss of power of recall in the memory tests, and a striking reduction in the rate of alternation of phases in the 'windmill' illusion. The last-named, indeed, is claimed as a very delicate index of drug effects, the writers ascribing the reduced rate of phase alternations to a rise in the resistance offered by the synapses of the neurons to the passage of the nervous impulse. Opium acts in a directly opposite manner. It accelerates the rate of phase alternations in the 'windmill' illusion, while it diminishes

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the errors in the dotting and in the memory tests, the attention being more easily directed to the task and to the meaning of the words learnt, and the process of recall being likewise facilitated.

Applying the dotting and other tests to ascertain the influence of food taken with alcohol, the writers find little subjective or objective effects when alcohol, to an amount of 30 c.c., is taken with a meal, whereas when it is taken from two to five hours after a meal the effects are unquestionable in the tests employed. Applying the dotting test to determine the effects of the degree of dilution of alcohol, they conclude that the weaker the solution the less marked are its effects. Both these results are in agreement with those of Dr. Mellanby (Report No. 31 of this series) and of Dr. Vernon (Report No. 34), but they call for further research.

A more extended investigation is also necessary in the light of the authors' interesting discovery that in the course of the protracted fatigue effects following several nights' loss of sleep, alcohol acts deleteriously during the stages of increasing inefficiency, whereas it acts beneficially as the subject later begins to regain his previous efficiency. At the former stage it increases the errors, at the latter it reduces the errors made in the tests. This suggests that an important cause of the conflicting results of past workers may be due to the stage or degree of fatigue when alcohol was taken.

MEDICAL RESEARCH COUNCIL,
15 BUCKINGHAM STREET,
STRAND, LONDON, W.C. 2.

September 1, 1920.

THE EFFECTS OF ALCOHOL AND SOME OTHER DRUGS DURING NORMAL AND FATIGUED CONDITIONS.

By William McDougall, F.R.S. and May Smith, M.A. (*from the Psychological Laboratory, Oxford*).

CONTENTS

		PAGE
I.	General Introduction	6
II.	The 'Dotting Machine' as a means of measuring the Effects of Alcohol, Opium, and Strychnine	7
	(*a*) Alcohol during Normal Conditions	7
	(*b*) Alcohol during the Fatigued State resulting from Loss of Sleep	10
	(*c*) The Effect of Time Intervals and Control Mixtures	13
	(*d*) Opium during Normal Conditions	14
	(*e*) Opium during Fatigue	15
	(*f*) The Curve of Recovery after Opium	16
	(*g*) Strychnine	17
III.	'Memory for Related Words' as a means of measuring the Effects of Alcohol, Opium, and Strychnine	18
	(*a*) Alcohol during Normal Conditions	19
	(*b*) Alcohol during Fatigue	19
	(*c*) Opium	21
	(*d*) Strychnine	21
IV.	Combination of the 'Dotting Machine' and 'Memory for Related Words' as a measure of the Effects of Alcohol and Opium	22
	(*a*) Alcohol	22
	(*b*) Opium	22
V.	The 'Perception of Ambiguous Figures' as affected by Alcohol and other Drugs, Tea, and Fatigue	24
	(*a*) Alcohol	25
	(*b*) Chloroform	26
	(*c*) Strychnine	27
	(*d*) Tea	28
	(*e*) Opium	28
	(*f*) Potassium Bromide	28
	(*g*) Fatigue	29
	Summary	29
VI.	Subjective Effects of the Drugs	30
	(*a*) Alcohol	30
	(*b*) Opium	30
VII.	Some Practical Applications to the Study of Alcohol	31
	(*a*) The Effect of the Degree of Dilution	31
	(*b*) The Effect of a preceding Meal	32
VIII.	General Summary	34

I. General Introduction.

The effects of alcohol, as expressed in terms of physiological or mental change, have been the subject of many experiments,[1] while in the public press and elsewhere medical men and others have defended diametrically opposite views as to its action, views apparently equally supported by facts.

The present investigation was undertaken primarily with the object of:

(1) Finding tests which would measure objectively the effects of drugs.

(2) Measuring the effects of moderate doses of alcohol and other drugs on the normal state; by the normal state is understood the general physiological conditions resulting at a given time of day from one's customary work.

(3) Determining the effect of the same quantities of alcohol and other drugs on the state supervening on considerable loss of sleep.

The following control precautions were taken:

(1) Daily records for many weeks were obtained, so that a comparison could be made between the drug effect, if any, and (*a*) the record for the day, (*b*) the weekly average.

(2) In order to ensure that the differences, if present, were due to the drug and not merely to the interval elapsing between two sets of experiments, the effects of time intervals, viz. 30 minutes, 60 minutes, were obtained for a period of about 12 months.

(3) The drugs forming the subject of the inquiry were all disguised[2] by one of us, so that one subject was in ignorance both as to their nature and number in the case of all the experiments dated 1913, 1914. Except where there is a statement to the contrary the writers were the subjects of the experiments.

(4) Mixtures containing no drugs, but resembling the drug mixtures in taste and appearance, were taken at irregular intervals, and so formed a control group.

(5) In the case of those mixtures which had marked physiological and subjective effects, one subject had no means of knowing that several might not have like effects.

(6) Prior to the investigation one subject had never to her knowledge taken alcohol in any form.

(7) The same time of day, viz. between 2 p.m. and 7 p.m., was as far as possible reserved for all the experiments. The usual method was to obtain a record of the particular test before taking the drug, then to take the drug, and after an interval obtain other records.

After about one hundred experiments with mixtures containing either caffein, potassium bromide, ethyl alcohol, tincture of opium, strychnine, or the control mixture, it was found that the first two

[1] Cf. Rivers, *Influence of Alcohol and other Drugs on Fatigue*; London, 1907; Dodge and Benedict, *Psychological Effects of Alcohol*, 1915.

[2] The disguise was usually effected by mixing small quantities of quinine, oil of peppermint, and coffee with the solution to be taken.

had no measurable effect on the subjects, while the alcohol and opium had such consistently marked effects, both subjectively and objectively, that future experiments in the case of one of us were chiefly concerned with a more detailed investigation of these two, and a few experiments with strychnine.

II. The 'Dotting Machine' as a means of measuring the Effects of Alcohol, Opium, and Strychnine.

The method was devised by one of us (McD.) as a test of sustained voluntary attention as long ago as the year 1900. The apparatus used in our experiments was the much improved form made by the Cambridge Scientific Instrument Co. at the suggestion of Dr. Rivers. It is essentially a 'mechanical device whereby a continuous band of paper tape, about one inch wide, is drawn behind an opening, or window, in the top of the desk, by a weight-driven clockwork movement'. Along the width of the band small red circles are distributed in as irregular a manner as possible, but at equal intervals. The test consists in marking the red circles with a stylographic pen as they pass before the subject's field of vision.[1] For these experiments a length of tape six metres long was the standard. This consisted of 1,200 small red circles, and the machine was so adjusted that the circles passed before the field of vision at a uniform rate of 5·8 a second. In each metre fifteen circles at irregular intervals were coloured blue beforehand, and these, when they appeared, were not to be dotted, but instead, the right hand had to be lifted to avoid marking the circle, while with the left hand a key had to be tapped, which, being attached to a pen arrangement under the lid of the machine, marked the tape and thus showed if the key had been tapped. It was possible to get by this means a very fine test of one's power of inhibition.[2]

Errors were of four types:—

(*a*) A circle allowed to pass unmarked.

(*b*) A blue circle marked which ought to have been left unmarked.

(*c*) An extra dot inserted between two circles.

(*d*) Slight deviations in either a lateral or vertical direction from the circle aimed at.

In estimating the errors, *a*, *b*, and *c* counted as one error on each occasion, *d* counted as half an error, if less than 2 mm.

(*a*) *Alcohol during Normal Conditions.*

There are in all thirty-three records of the effect of alcohol on the ordinary normal state; by which is meant a state in which there was no fatigue except such as might be assumed to be present normally at such a time of day.

[1] Burt, 'Experimental Tests of General Intelligence,' *Brit. J. Psychol.*, 1909, 3, 153; Smith, 'A Contribution to the Study of Fatigue,' *Brit. J. Psychol.*, 1916, 8, 329.

[2] With subjects less practised than the writer at this particular test it is quite sufficient to have each circle marked simply without the complications described above. The subject (M. S.) of these experiments had been using this test as a fatigue test for several years.

The doses varied from 10 c.c. to 30 c.c. of absolute alcohol (mixed with three parts of water), but most of the experiments were done with doses of 15 c.c. or 20 c.c. The time of day given in the table is the time when the drug was taken.

With 10 c.c. there are ten experiments, the details of which are given below.

No.	Date.	Drug taken at	No. of Errors immediately before taking Alcohol.	No. of Errors one hour[1] after taking Alcohol.
1.	24 June, 1913	5.15 p.m.	79	115
2.	3 July, 1913	5.30 p.m.	81	63
3.	13 July, 1913	5.45 p.m.	84	113
4.	20 July, 1913	6.30 p.m.	76	106
5.	25 July, 1913	5 p.m.	83	84
6.	27 July, 1913	6.10 p.m.	79	111
7.	22 Nov., 1913	5 p.m.	71	89
8.	28 Nov., 1913	4.30 p.m.	80	102
9.	3 Dec., 1913	5.20 p.m.	89	83
10.	31 March, 1914	4.50 p.m.	82	107
			Mean: 80·4	Mean: 97·3

21 per cent. increase in the number of errors.

It will be noticed that in eight cases, there is an increase in the number of errors after taking the drug, although in one case so slight as to fall within normal variation.

In the two remaining cases, viz. 2 and 9, alcohol is followed by a decrease in the number of errors, in one case so small as to fall within normal variation, in the other case a normal of 81 is followed by 63, a result better than normal. The subjective comment made before performing this experiment is 'very tired'; in the later experiments, showing the effect of alcohol on a fatigued state, we may get some explanation.

There are eleven experiments with 15 c.c. alcohol, and of these in ten cases there is an increase in the number of errors, though in some cases a very slight one.

No.	Date.	Drug taken at	No. of Errors immediately before taking Alcohol.	No. of Errors one hour after taking Alcohol.
1.	28 June, 1913	5 p.m.	54	77
2.	6 July, 1913	6.10 p.m.	80	177
3.	17 July, 1913	5.15 p.m.	85	189
4.	22 July, 1913	4.35 p.m.	76	97
5.	23 Nov., 1913	6 p.m.	93	128
6.	6 Dec., 1913	6 p.m.	68	112
7.	12 Dec., 1913	4.10 p.m.	78	54
8.	21 Feb., 1914	5.10 p.m.	102	106
9.	3 March, 1914	3.15 p.m.	87	109
10.	29 May, 1914	4.15 p.m.	57	77
11.	2 June, 1914	3 30 p.m.	73	76
			Mean: 77·5	Mean: 109·2

42 per cent. increase in number of errors.

In one case, No. 7, there is improvement after alcohol, viz. only 54 errors.

[1] The original time interval of one hour was chosen for practical purposes as these experiments had to be arranged during leisure time. Later an attempt was made to adjust the claims of our other work to these experiments, but such was not always possible.

With 20 c.c. there are nine experiments, eight of which are followed by an increased number of errors after alcohol, No. 6 being the exception.

No.	Date.	Drug taken at	No. of Errors immediately before taking Alcohol.	No. of Errors one hour after taking Alcohol.
1.	18 June, 1913	5.5 p.m.	61	114
2.	1 Dec , 1913	3 p.m.	87	160
3.	7 Dec., 1913	6 p.m.	72	154
4.	10 Dec., 1913	3.10 p.m.	53	57
5.	16 Dec., 1913	11.10 a.m.	50	63
6.	18 Dec., 1913	4.45 p.m.	63	54
7.	7 Feb., 1914	6.25 p.m.	41	69
8.	13 Feb., 1914	4.20 p.m.	110	117
9.	15 May, 1915	6 p m.	93	147
			Mean : 70.0	Mean : 97·2

39 per cent. increase in the number of errors.

It will be noticed that with this dosage in one case, No. 6, there is a decrease in the number of errors after the drug, whereas in the other cases there is a considerable increase except in No. 4 and No. 8, which would fall within normal variation.

With 25 c.c. only three experiments were made, and in each case there is an increase in the number of errors made after taking alcohol.

No.	Date.	Drug taken at	No. of Errors immediately before taking Alcohol.	No. of Errors one hour after taking Alcohol.
1.	8 Dec., 1913	4.50 p.m.	75	122
2.	27 Feb., 1914	3.50 p.m.	75	148
3.	23 May, 1914	2.20 p m.	81	223
			Mean : 77	Mean : 164

113 per cent. increase in the number of errors.

It has been mentioned above that it is possible to have several types of error. In the tables all types are pooled as the effect of the alcohol is shown by a proportional increase of all types, i. e. in extra dots inserted between the circles as well as in ineffective shots falling short of the circle.

After such doses as are represented in these experiments, alcohol seems in the majority of cases to be followed by a marked loss of accuracy and of control, the latter being illustrated by the increase in the number of insertions.

A quite common subjective effect, which accompanied many of the alcohol experiments was a pleasant conviction that the dotting was very good; in fact that particular view persisted long after the delusive nature of the experience was well known, and it frequently crossed the subject's mind that in this or that particular case alcohol was not having its usual effect, only to find the customary increase in the errors when they were counted. It will have been noticed that in several cases in the above experiments alcohol was followed by a slight improvement. Perhaps some light will be thrown on these when the influence of alcohol on a fatigued state is considered.

(b) Alcohol during the Fatigued State resulting from Loss of Sleep.[1]

The effect of alcohol during the fatigued state was incidental to an investigation on fatigue which had for one of its aims to trace the nature of the recovery curve after the fatigue ensuing from a voluntary cutting down of the normal amount of sleep for three nights.[2] The effect of loss of sleep, as tested by the dotting machine, showed itself in two distinct phases:

(1) A phase of about three days' duration when fatigue acted apparently as a stimulant, so that the errors were fewer than in the normal state.

(2) A phase of longer duration, about thirteen days, when the errors gradually rose to a number considerably higher than normal, followed by a somewhat irregular return to the normal.

The effects of alcohol were observed in the course of four 'fatigue cycles'.[3] To avoid the possibility of the subject becoming accustomed to a certain amount of the drug, no attempt was made to get daily records, and in fact during the first of these four cycles only six days out of the 23 during which fatigue conditions prevailed, were alcohol days; on the other days either other drugs or a mixture disguised as a drug had been taken.

In the last of the four the investigation was confined to the effects of alcohol, and as it contains more experiments than the previous ones it has been given in detail. On this occasion the subject knew that alcohol was being taken, but this knowledge did not give results in conflict with the earlier ones done in ignorance of the drug.

The dosage was on each occasion 15 c.c., and the experiments began at 2.30 p.m. each day. As a result of one night with only one and a half hours' sleep, the errors in the dotting fell from an average of 52 for the preceding fortnight to 38; half an hour after having taken 15 c.c. alcohol the errors rose to 137, a number greater than any resulting from fatigue during this 'cycle'.

Three days later alcohol records were again obtained and the errors increased from 45 before to 113 after alcohol. Daily records were obtained in order to trace the fatigue curve, but alcohol records only on a few days.

Four days later, by which time the second fatigue phase was in evidence, as shown by the rise of the errors to 109, alcohol increased this number to 161, and for several days there were similar records (see Graph I).

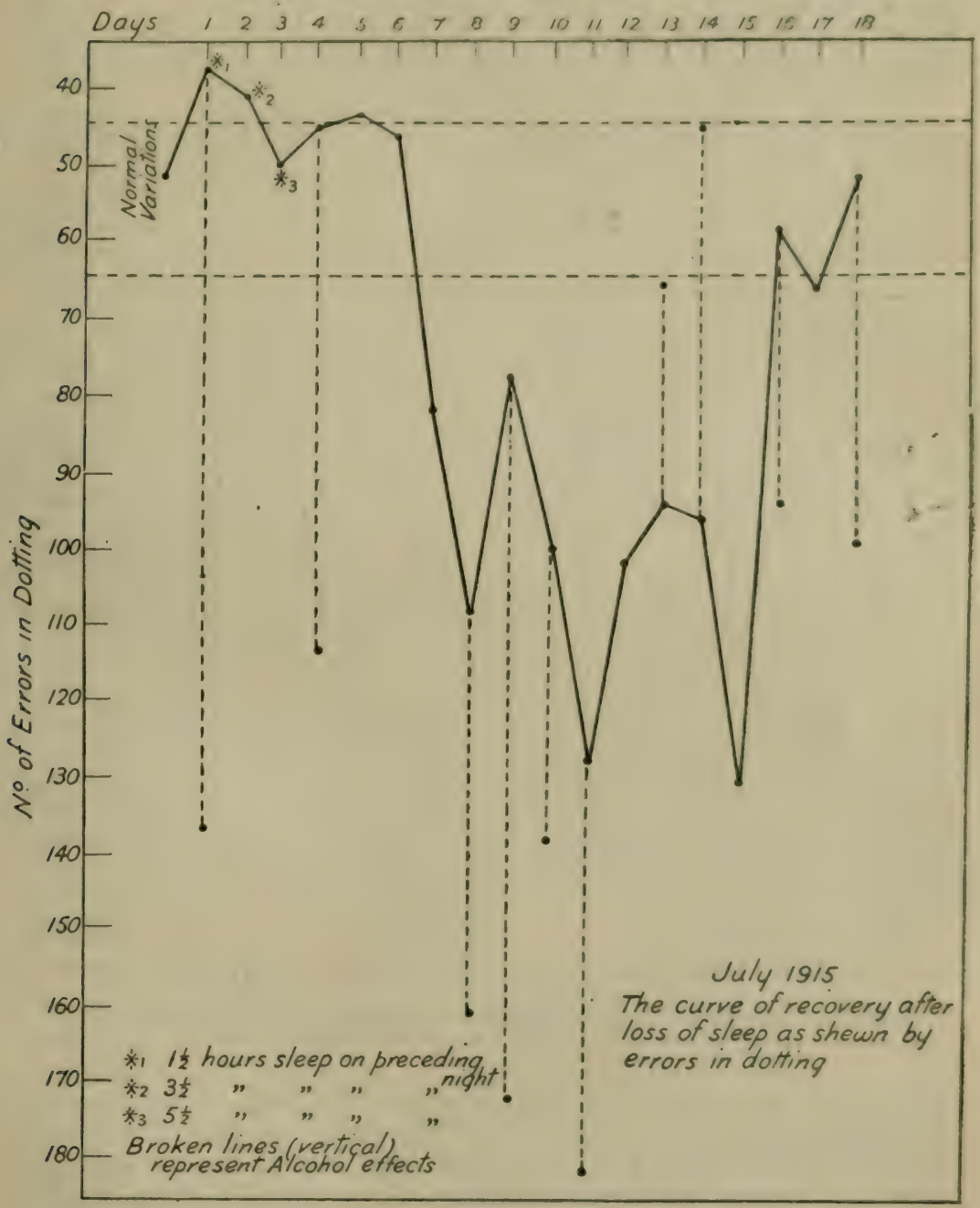
Towards the end of the fatigue cycle, however, when the graph shows a tendency to approach the normal, the alcohol reaction is different; for on the thirteenth day the errors in dotting being 95, the effect of alcohol is to reduce the errors in dotting to 66, with a similar result on the following day, viz. 96 errors in

[1] Throughout this paper the word 'fatigue' is used for the effect of loss of sleep. In order to keep awake for the pre-arranged number of hours the subject (M. S.) worked to a time-table, drawn up beforehand, and the work done was as near as possible to that of the usual daily routine.

[2] For full details of the fatigue experiments see *Brit. J. Psychol.*, 1916. 8. 327.

[3] By 'fatigue cycle' is meant the period covered by the actual loss of sleep and the return to a normal. The whole generally occupied from 16 to 25 days.

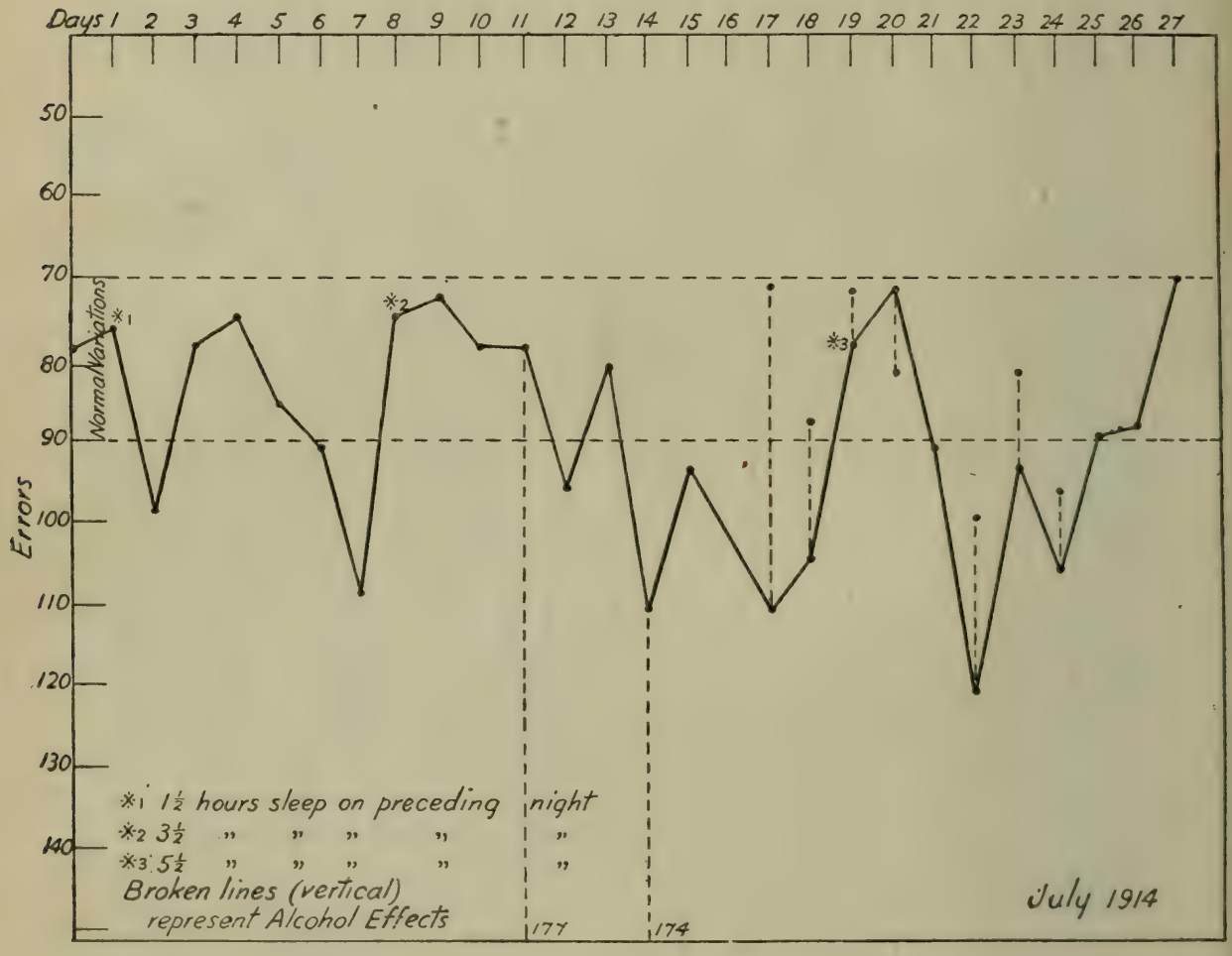
dotting before alcohol is followed by 45 after alcohol. This improvement might be interpreted as perhaps representing a certain habituation to the drug; such an hypothesis, however, is ruled out by the return two days later to a normal number of errors followed by the more frequent alcohol reaction, viz. 57 before alcohol followed by 95 after, and two days later 51 before is followed by 100 after.



GRAPH I. Showing the effects of alcohol at various stages of a 'fatigue cycle'.

This decrease in the number of errors towards the approach of the return to normality occurs in some of the 'fatigue cycles' prior to the one just described, e.g. twice in the November 1914 series, viz. on the fifteenth and nineteenth day, but as no alcohol experiments after these occur in the series, the records could not be regarded as conclusive, and in the cycle of July 1914 the same

phenomenon occurs several times from the sixteenth to the twenty-second day. This particular cycle differs somewhat from the others. The aim was to see what difference was made if, instead of cutting down sleep for three nights in succession, the same loss of sleep was spread out over a longer period, i.e. one night with only $1\frac{1}{2}$ hours' sleep was followed by a period of six days when more than the usual amount of sleep was taken; then a night with only $3\frac{1}{2}$ hours' sleep occurred, followed by ten ordinary nights, when a third night of $5\frac{1}{2}$ hours' sleep was spent. Now there are two alcohol



GRAPH II. Showing the effect of alcohol on a 'fatigue cycle' when the loss of sleep occurred at intervals, and not on successive days.

records on the eleventh and fourteenth days, each of which gives the usual increase of errors after the drug, viz. from 78 to 111 before to 177 and 174 after respectively, but on the seventeenth day 111 errors before alcohol are followed by 71 after, on the eighteenth day 105 errors are followed by 88. These records occur between the seventh and tenth day from the second night of diminished sleep; at this stage occurred the third night of shortened hours of sleep with the result that the number of errors falls from 105 on the day before to the normal 77, which is followed by 73 half an hour after taking alcohol; on the next day 71 errors before alcohol is followed by 81 after, after which for three days alcohol is followed by a slight improvement, viz. 121, 93, and 107

before alcohol are followed by 104, 82, and 91 respectively after[1] (see Graph II).

Alcohol seems thus to have a different effect according to the stage of recovery from fatigue and breaks up the curve of recovery again. It has been shown before that after considerable loss of sleep, tested by the power to give sustained voluntary attention, there is a stage when fatigue acts as a stimulant followed by a stage characterized by deterioration in the powers tested. This second phase is, according to the alcohol experiments, again divided off into two parts, (*a*) the stage when alcohol has the same effect as on the normal state, i.e. deteriorates the higher powers; (*b*) the stage when it seems to act as a stimulant and improves the work done.

It will have been noticed that in describing the effects of alcohol on the normal state a few occasions occurred when a decrease in the number of errors occurred after alcohol; in these cases the subjective statement written before doing the experiments is, with one exception, when there is no comment whatever, 'very tired', and in no case does this statement occur in the other records. Unfortunately these experiments do not determine what exactly is the degree of fatigue which can be neutralized by alcohol, for at a time when the fatigue is most acute the alcohol reaction is the more usual one.

If these results are not purely personal, then probably one reason for the very uncertain and contradictory results that investigators record with alcohol is the varying condition of the body with regard to fatigue.

Wherever regular records are available for the same person for any one test, daily fluctuations, sometimes considerable, are sure to be observed, fluctuations which, however, when they are analysed, are not as erratic as they at first appear. A long series of drug experiments is necessary before variations can without reserve be attributed to the drug. Also we might argue that the test used did not permit of much improvement, so that a drug could only affect it in one direction. Such a view must, however, consider the effect of other drugs. Some light, therefore, on the alcohol results may be thrown by the study of other drugs as, e.g. opium, and also by the combination of drugs.

(*c*) *The Effect of Time Intervals and Control Mixtures.*

In order to be sure that the differences apparently taking place after the drug were due to the drug and not merely to the length of time which had elapsed between the two sets of experiments, a series of experiments was made with the dotting machine test to get the effect of the time interval. These did not all take place in a daily sequence, but were interspersed among the drug experiments and extended over a period of about twelve months. There are 15 records of dotting done at intervals of 30 minutes; of these 9 show an increase of errors, 5 a decrease, and 1 no change, the average being from 78 before to 87 after. There are 25 records at intervals of one hour, of which 14 show an increase in the number of

[1] Some of the improvements are so slight that they certainly would not be considered as drug effects were it not for the fact that normally the alcohol reaction would have been to increase the errors considerably, so that a relatively slight decrease may represent a considerable effect. Cp. the effect of mixing two drugs of opposite actions, p. 27.

errors and 11 show a decrease, the average of all being an increase from 88 to 90 errors. In two of the experiments at intervals of one hour, however, there is a marked difference, viz. from 75 to 112 errors, and from 72 to 101 errors. Had a drug been taken on these days the difference would doubtless have been ascribed to it. The subjective comment for the first of these is, 'very tired'. Under ordinary circumstances the difference in dotting errors for any period between 2 o'clock and 6 o'clock is very slight; when, however, the subject was fatigued to begin with, it sometimes happened that the errors were not increased for the earlier part of the afternoon, but that the fatigue showed itself later in an increased number of errors, and invariably on the following day; and this is the interpretation of these unusual variations.

With regard to those mixtures which purported to contain drugs but which actually did not, there are 20 records, of which 9 show an increase of errors and 11 a decrease, the average being from 78 before to 79 after. Contrast these variations with those occurring after alcohol.

(*d*) *Opium during Normal Conditions.*

The effects of alcohol, as determined by objective tests, are thrown into relief by a consideration of the effects of opium and some other drugs here considered.

Dosage.—The doses varied from 40 minims to 90 minims of the tincture of opium, and the experimenters were the writers.

Time Interval.—It was found by experiment [1] that the maximal effect of opium was obtained between 75 minutes and 115 minutes after taking the drug. In the earlier experiments the same time interval, viz. one hour, was allowed for all drugs, but this proving too short, in the experiments here recorded a longer interval was allowed.[2]

There are eight experiments with doses of 40 minims, and of these all but one are followed by a noticeable decrease in the number of errors. In the case of the exception the interval was under one hour.

Details of the Experiments.

40 Minims.

No.	Date.	Drug taken at	No. of Errors immediately before taking the Drug.	Errors after taking the Drug.
1.	July 18, 1913	5.35 p.m.	100	72 (interval: 1 hour)
2.	July 21, 1913	5.15 p.m.	75	74 (interval too short: 40 mins.)
3.	July 24, 1913	5.10 p.m.	71	55 (interval: 1 hr.)
4.	Dec. 17, 1913	4.20 p.m.	85	43 (,, 1½ hrs.)
5.	March 13, 1917	2 p.m.	62	44 (,, 1 hr. 45 mins.)
6.	March 15, 1917	2.15 p.m.	82	40 (,, 1 hr.)
7.	March 22, 1917	4·40 p.m	102	68 (,, 1 hr. 40 mins.)
8.	March 25, 1917	4.55 p.m.	64	42 (,, 1 hr. 20 mins.)
			Mean: 80·1	Mean: 54·7

31·6 per cent. decrease in the number of errors.

[1] This was done by getting dotting records every five minutes for a period of two hours, but unfortunately circumstances did not permit the experiment at the beginning of the research.

[2] Although desirable in the interests of scientific research that the time intervals for all experiments should be always the same, yet in work that extends over some years, and has to be done in leisure time, it is found that the demands of practical life make such rigidity impossible.

There are 24 experiments with doses of 60 minims and in 21 cases the normal number of errors is followed by a decrease in the number of errors after opium: in the three exceptions there is a slight increase in the errors. In one case, No. 4, 90 minims had been taken on the preceding day.

60 Minims.

No.	Date.	Drug taken at	No. of Errors immediately before taking the Drug.	Errors after taking the Drug.
1.	Feb. 18, 1914	3.7 p.m.	68	48 (interval: 40 mins.)
2.	April 5, 1914	2.45 p.m.	64	49 (,, 1 hr.)
3.	May 6, 1914	2.25 p.m.	73	59 (,, 1½ hrs.)
4.	May 15, 1914	6.30 p.m.	84	87 (,, 1 hr. 25 mins.)
5.	May 22, 1914	2.35 p.m.	89	101 (,, 2 hrs.)
6.	Oct. 13, 1914	2.30 p.m.	80	61 (,, 1 hr. 45 mins.)
7.	Oct. 14, 1914	3.30 p.m.	48	43 (,, 2 hrs. 30 mins.)
8.	Oct. 15, 1914	2.20 p.m.	85	50 (,, 2 hrs.)
9.	Oct. 17, 1914	5.20 p.m.	54	49 (,, 1 hr. 35 mins.)
10.	Oct. 19, 1914	3.35 p.m.	73	48 (,, 2 hrs. 15 mins.)
11.	Oct. 20, 1914	3.50 p.m.	70	64 (,, 2 hrs. 15 mins.)
12.	Oct. 21, 1914	2.30 p.m.	70	61 (,, 1 hr. 30 mins.)
13.	Oct. 27, 1914	3.30 p.m.	72	53 (,, 2 hrs.)
14.	Oct. 29, 1914	4.5 p.m.	73	52 (,, 2 hrs.)
15.	Nov. 3, 1914	3.30 p.m.	68	57 (,, 2 hrs.)
16.	Nov. 4, 1914	2.25 p.m.	52	35 (,, 1 hr. 50 mins.)
17.	Dec. 9, 1914	3.55 p.m.	64	40 (,, 1 hr. 50 mins.)
18.	Nov. 22, 1915	3.30 p.m.	84	76 (,, 3 hrs.)
19.	May 18, 1916	3 p.m.	80	50 (,, 2 hrs. 30 mins.)
20.	April 24, 1917	3 p.m.	76	40 (,, 1 hr. 30 mins.)
21.	May 7, 1917	4 p.m.	70	82 (,, 2 hrs. 30 mins.)
22.	May 12, 1917	4.55 p.m.	108	50 (,, 1 hr. 30 mins.)
23.	June 21, 1919	1.40 p.m.	98	84 (,, 3 hrs.)
24.	June 26, 1919	3.30 p.m.	106	62 (,, 2 hrs. 30 mins.)
			Mean: 75.4	Mean: 58.4

22.5 per cent. decrease in number of errors.

There are only a few experiments with doses of 80 and 90 minims—too few to be of any statistical value.

With such doses of opium the general result is that a test which demands for its successful execution concentrated voluntary attention is in the majority of cases improved, a result in marked contrast to the effect on this test of alcohol. We cannot compare the effect of increasing the dosage as there are too few experiments with the smaller amount.

A marked subjective effect was the feeling that the machine was going less quickly, so that there seemed to be more time to 'dot' easily. The dotting was also neater.

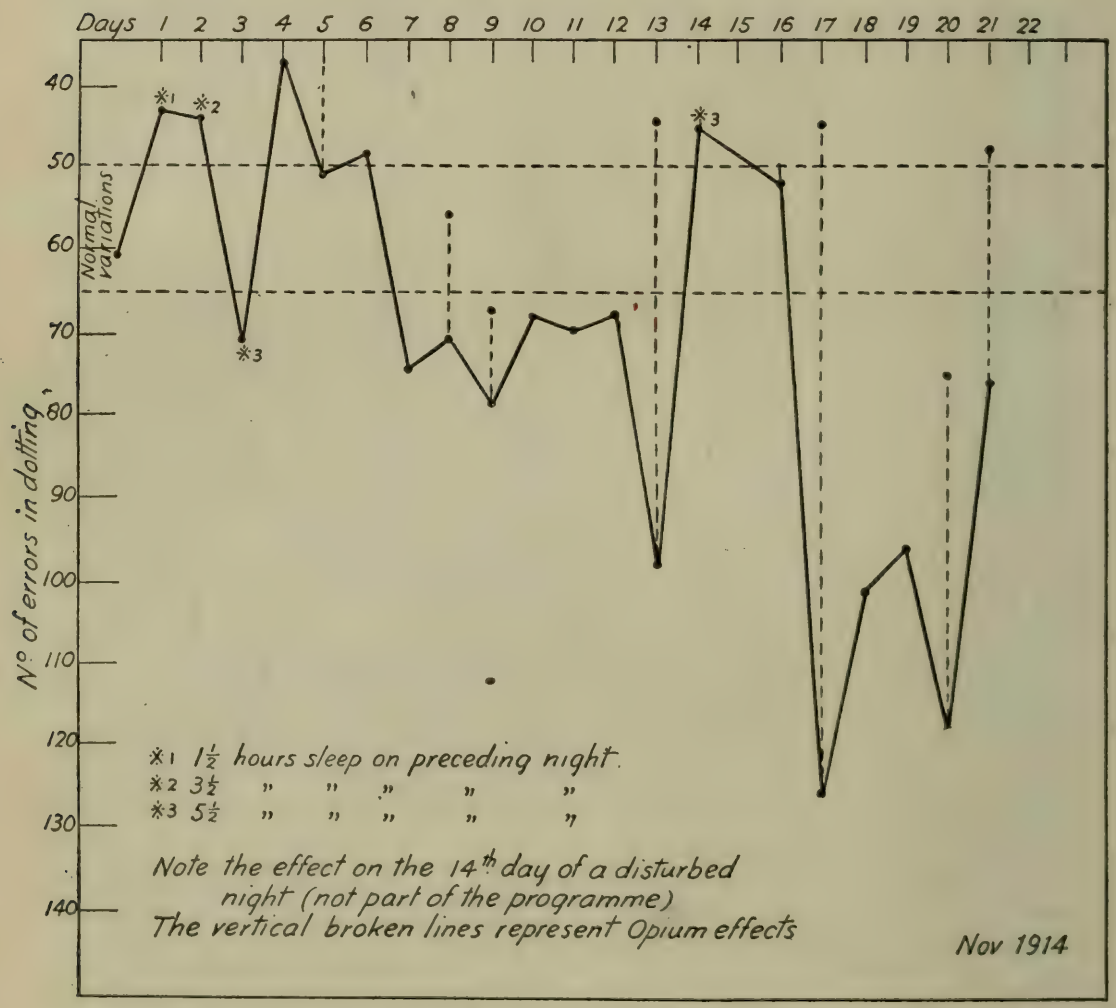
(*e*) *Opium during Fatigue.*

The conditions for determining the effects of opium on a fatigued state were similar to those described above for alcohol, but it was not thought desirable to include so many doses. As it gave the same results in several 'fatigue cycles', the results have been pooled, as no one fatigue cycle contains sufficient experiments.

There are sixteen records of opium effects at various stages of a fatigue cycle, viz. (*a*) during the actual period of shortage of sleep, (*b*) during the earlier stage of recovery, when, as shown in a previous article,[1] tasks involving either memory or

[1] Smith, 'A Contribution to the Study of Fatigue', *Brit. J. Psychol.*, 1916, 8, 329.

attention are considerably deteriorated, and (*c*) during the later stage of recovery immediately preceding the return to normal, when alcohol has the effect of improving work. Seven of these records occur during phase (*a*), and the effect of the opium in all cases is to improve the dotting, so that an average of 77 errors before the drug is followed by an average of 63 errors after. Nine records occur during phase (*b*) and (*c*) which, however, are not differentiated by the opium; the results are the same as in the earlier phase, but more exaggerated in proportion, as the initial records show the deteriorating effect of fatigue, i.e. an average of 110 errors before taking the drug is followed by an average of 73 errors after. See Graph III.



Graph III. Showing the effect of opium at various stages of a 'fatigue cycle'.

(*f*) *The Curve of Recovery after Opium.*

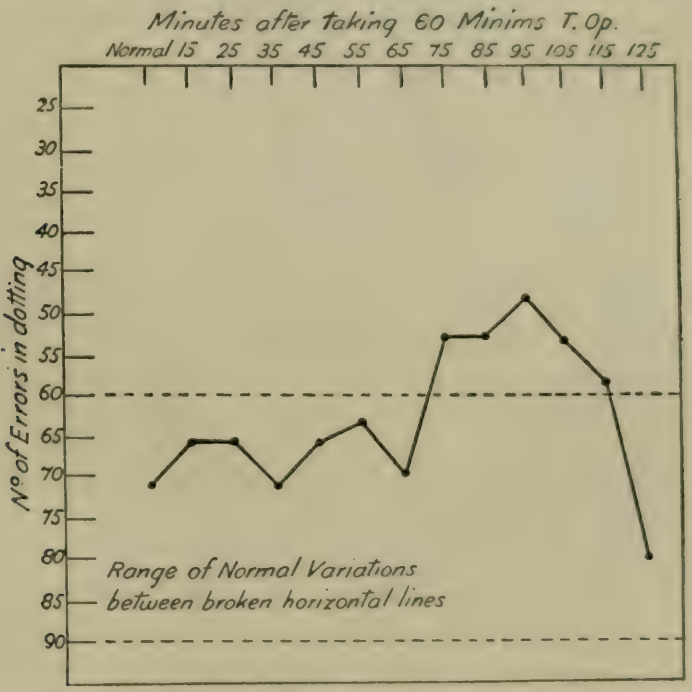
Several experiments were devoted to getting records every ten minutes, with the object of tracing the stages of the disturbance effected by opium.

The experiment that proved most suitable for such work was the dotting machine.

The procedure was to get a normal record on each occasion, then to take the drug, then to allow an interval of fifteen minutes,

after which another record was obtained, and then to obtain similar records every ten minutes. There are eight experiments and the averages of the variations for every ten minutes are given. This form of the experiment was not adopted until April 1917.

An average of 72 (extremes 60 and 90) before the drug is followed by an average of 66 fifteen minutes after, which improvement may be only a normal variation; after this normal records are obtained until 75 minutes have elapsed since the drug was taken, and the records are superior to the normal until 115 minutes have elapsed, after which there is a return to the normal. It was never possible during this series of experiments to prolong the records past this length of time, so that we have no evidence as to whether the drug has a deteriorating effect later, nor whether the normal apparently reached is reliable. Some of the experiments given above show opium effects over a longer period. See Graph IV.



GRAPH IV. Showing the effect of opium at intervals of 10 minutes.

The maximum effect of the drug as illustrated by this test is, then, between 75 and 115 minutes after taking it. Subjectively the symptoms were pronounced after about 20 minutes and frequently persisted in a vague way for several hours. Subjective variations were only noted if they were obtrusive, as it is easy to imagine symptoms if one is looking out for them.

One record traced the effect of a hypodermic injection of $\frac{1}{4}$ gr. morphine hydrochlor. In this case the effects are shown within 15 minutes, and are much more exaggerated. See Graph V.

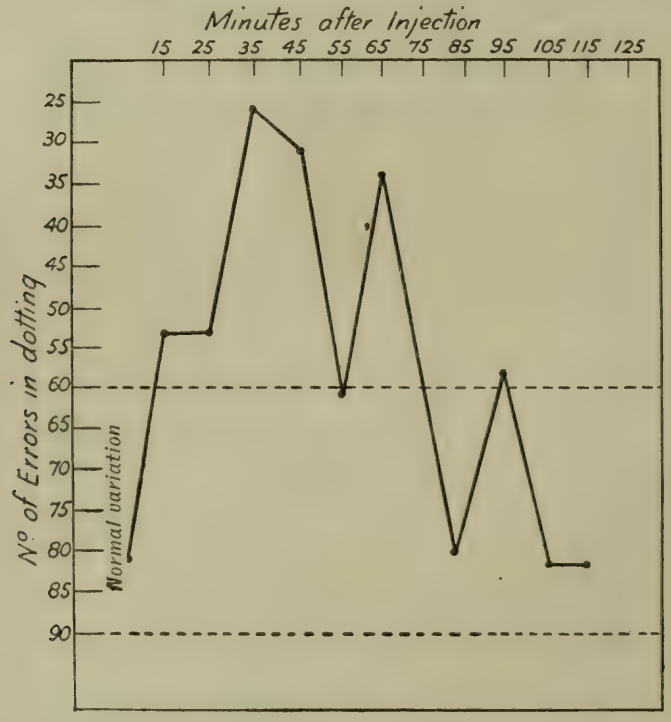
(*g*) *Strychnine.*

There are only five records of doses of strychnine varying from $\frac{1}{15}$ gr. to $\frac{3}{15}$ gr. taken by the mouth. These were done by one subject (M.S.) who was in ignorance of the drug (if any) taken. The five experiments do not occur together, but are separated by considerable intervals. The results are similar; in every case there is an

improvement after the drug, although in one case the difference is not significant. No attempt was made to get the effect of strychnine on a fatigued state, nor did we continue with this drug.

Date.	Drug taken at	No. of Errors immediately before taking Str.	No. of Errors after Str.	Dosage.	hr.	min.
Feb. 12, 1914	4 p.m.	139	104	$\frac{1}{15}$ gr. St. Nit.	interval	30
,, 17 ,,	3.20 p.m.	106	94	$\frac{2}{15}$ gr. ,,	,, 1	5
,, 20 ,,	3 p.m.	112	92	$\frac{2}{15}$ gr. ,,	,, 1	0
,, 26 ,,	3.50 p.m.	89	84	$\frac{1}{15}$ gr. ,,	,, 1	30
Apr. 3 ,,	3.20 p.m.	88	79	$\frac{2}{15}$ gr. ,,	,, 1	30
		Mean : 106·8	Mean : 90·6			

15 per cent. decrease in number of errors.



Graph V. Showing the effect of a hypodermic injection.

III. Memory for Related Words as a means of measuring the Effects of Alcohol, Opium, and Strychnine.

This test in the form used was devised by one of us. The test required (*a*) that the subject should appreciate a relationship between words in sequence, and (*b*) be able to reproduce the words in the right sequence. A list of forty words drawn up by a collaborator was read to the subject; the list was so arranged that there was a connexion between the first word and the second, between the second and the third, and so on, a connexion which the subject could grasp by an act of attention, e.g. mountain, plain, ugly, beauty, Venus, Greece, oil, smooth, rough, refined, sugar, sweet, salt, sea, sky, angel, spirit, brandy, bottle, cork, &c.

The list was read to the subject at a uniform rate of one word in two seconds. After the list had been read through once, the

subject, having been told the first word, had to reproduce verbally the complete list in right sequence: failure to give the correct word within ten seconds counted as one error, in which case the collaborator gave the correct word and the subject proceeded: a wrong word corrected[1] by the subject within ten seconds counted as half an error.

(*a*) *Alcohol during Normal Conditions.*

With this test there are only three alcohol experiments under unfatigued conditions, as it did not form part of the original plan. Under normal conditions the average number of errors in a list of forty words was 7 (extremes 5 and 9); after 15 c.c. alcohol the errors rise to 20, 16, and 20½ respectively for the three experiments.[2]

Analogous to the belief that the dotting was very good was the conviction (almost invariably present after the drug, which proved to be alcohol), that the particular list was very easy, and that no difficulty would be experienced in reproducing it correctly, a comfortable conviction soon shattered when put to the test.

During the period following loss of sleep, when one is trying to make up for previous strain by extra sleep, one's attention in doing this test is apt to wander, so that (*a*) many of the sequences are not heard with meaning, i. e. they just affect one as auditory sensations; (*b*) in some cases the word is heard and part of its significance grasped, but not its relation to the preceding or succeeding word, a very necessary condition for success in this particular test; it is to this failure of attention that many of the errors due to fatigue are made. Now in the case of alcohol there was no failure to attend to the list, each sequence was heard and comprehended, hence the feeling that the list was easy; at the conclusion of the reading the whole list seemed to be present to consciousness for a moment, and so one felt assured of one's perfect success in reproduction; as soon, however, as the signal was given by the collaborator saying the first word, the whole seemed dramatically to be wiped out, and the number of errors ensuing always astonished the subject.

(*b*) *Alcohol during Fatigue.*

The general conditions have been already described in the section devoted to the 'dotting' experiments. During several fatigue cycles this test was used with similar results, so only one is given in detail.

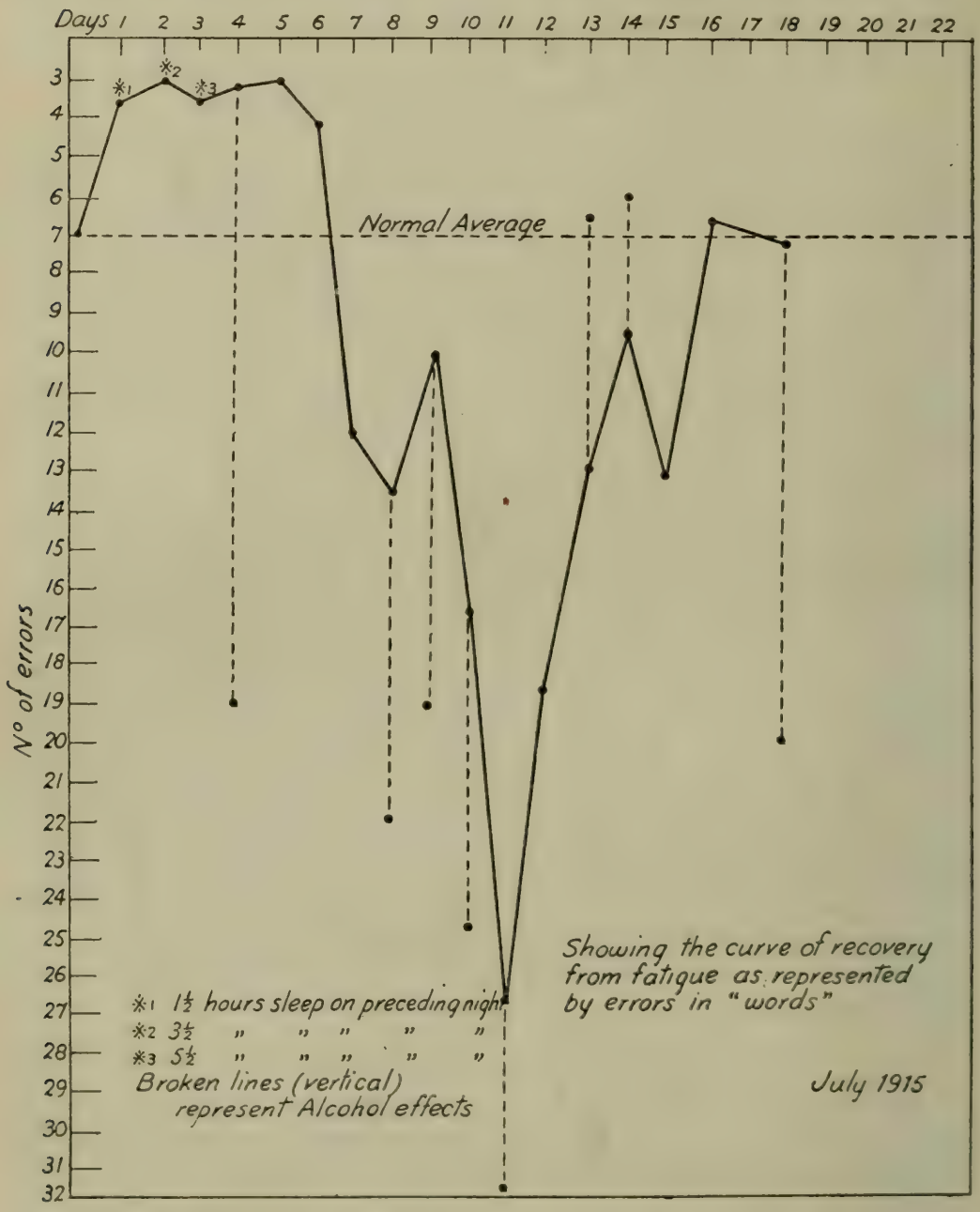
The dosage was in each case 15 c.c. and the time of day for each experiment 3 p.m.

As a result of one night of 1½ hours' sleep the errors in words fall from an average of 7 for the preceding fortnight to 3½; half an hour after having taken 15 c.c. alcohol the number of errors is 21. Three days later alcohol records were again obtained and the errors increase from 3 before the drug to 19 after. Four days

[1] A list unknown to the subject has of course to be used for every experiment. As lists vary in interest and difficulty the list is not quite as useful as the 'dotting', but the subjects of these experiments had had so many experiences of the test that the number of errors was found to be very regular under normal conditions.

[2] The dates are from May 28 to June 3, 1919, between 4 p.m. and 6 p.m.

later, by which time the second fatigue phase was in evidence, as shown by the rise in errors to 13½, after alcohol the errors rise to 22, and for several days there are similar records (see Graph VI).



GRAPH VI. Showing the effect of alcohol at various stages of a fatigue cycle as represented by the number of errors in lists of 'words'.

On the thirteenth day, however, when the graph shows a consistent tendency to approach the normal, the alcohol reaction is different, for the errors in the words decrease after the drug from 13 to 6½, with a similar result on the following day, viz. 9½ errors before is followed by 6 errors after. This effect, as stated previously, cannot be interpreted as representing a certain habituation to the drug, for on the eighteenth day, when the effect of the loss of sleep had worn off, the normal number of errors of 7 is followed after alcohol by the normal alcohol reaction, viz. increase to 20.

The related words test thus gives a result similar to the 'dotting' test, viz. breaks up the second fatigue phase into two stages, (*a*) the stage when alcohol has the same effect as on the normal state, i.e. deteriorates the higher powers as represented by attention and memory; (*b*) the stage when it seems to act as a stimulant and improves the same powers.

(*c*) *Opium.*

As this test was added after many experiments with the dotting machine, there are fewer records and none with 40 minims. With 60 minims there are 11 records and in 10 of these there is a decrease in the number of errors, the exception being No. 2.

No.	Date.	Drug taken at	No. of Errors immediately before taking the Drug.	No. of Errors after taking the Drug.		
					hr.	min.
1.	May 22, 1914	2.35 p.m.	7	5 (interval:	2	5)
2.	Oct. 13 ,,	2.30 p.m.	7	8 (,,	1	50)
3.	Oct. 17 ,,	5.20 p.m.	5½	3 (,,	1	40)
4.	Oct. 21 ,,	2.30 p.m.	7	3 (,,	1	35)
5.	Oct. 27 ,,	3.30 p.m.	9½	0 (,,	2	5)
6.	Oct. 29 ,,	4.5 p.m.	14	2 (,,	2	5)
7.	Nov. 3 ,,	3.30 p.m.	9	4½ (,,	2	5)
8.	Nov. 4 ,,	2.25 p.m.	7	2½ (,,	1	55)
9.	Nov. 22, 1915	3.30 p.m.	7½	6½ (,,	3	5)
10.	Apr. 24, 1917	3 p.m.	12	1 (,,	1	35
11.	May 7 ,,	4 p.m.	5	2½ (,,	2	35)
			Mean: 8·2	Mean: 3·4		

58 per cent. decrease in number of errors.

In a few of the experiments the improvement is almost dramatic, the errors falling, as in No. 5, from 9 before to 0 after, a difference never occurring under normal conditions.

Another characteristic of the reproduction after opium is the speed with which the list is reproduced. The conditions of the test are, that the length of time allowed for the reproduction of the succeeding word is not to exceed ten seconds. Normally it takes 2½ minutes to reproduce the list; after opium the time taken is between 1 and 1·5 minutes, and the words seem to roll themselves off automatically, in marked contrast to the great difficulty experienced with alcohol in reproducing even those that are known. The subjective attitude, too, towards the list is different, for not only is the relationship between the successive words grasped, but when listening to the list the subject finds himself making tentative mental suggestions for the next word and noting the different relationship between the anticipated word and the actual word. Normally this rarely occurred, the two seconds allowed for the word being occupied in grasping the relationship.

Opium thus seems to improve the capacity to attend to and to reproduce sequences of related words.

(*d*) *Strychnine.*

There are only three experiments with this test, but the effect is the same in every case, viz. a distinct improvement. The average normal of these three cases is 5 errors; after the drug the errors are 0, 3, ½ respectively, thus giving a result akin to that of opium and opposed to the usual effect of alcohol.

IV. Combination of the 'Dotting' Machine and Memory for Related Words as a Measure of the Effects of Alcohol and Opium.

This test was originally used in order to test the reliability of that improvement which shows itself in the tests done separately as a result of considerable fatigue.[1] A collaborator read a list of fifty related words to the subject, while the latter was dotting the usual length of tape under the usual conditions. Read at the rate of one word in two seconds, the fifty words read twice lasted the same length of time as the dotting. At the completion of the dotting the list of words was reproduced and the errors counted in the usual way. In this case the problem is to perform together two operations, both of which demand concentrated attention.

(*a*) *Alcohol.*

There are only three alcohol experiments with this test, but the same reaction takes place in all, and the difference in the number of errors before and after alcohol is well marked. On the first occasion of the combined test with alcohol[2] the normal records are 51 errors in dotting and $8\frac{1}{2}$ in words;[3] half an hour after taking 20 c.c. alcohol the dotting errors are 164 and the errors in words 34. May 4, 1915, 3–4 p.m.

The next experiment, on May 6, results in the normals of 61 and and $9\frac{1}{2}$ for dotting and words respectively, being followed by 133 and 28 after 20 c.c., while in a third experiment, May 7, 1915, 69 dotting errors and 4 errors in words are followed by 93 dotting errors and $32\frac{1}{2}$ errors in words.

Alcohol thus affects the double process test similarly to the single tests and is analogous to the operation of fatigue when the period of stimulation is over.

(*b*) *Opium.*

This double test had proved to be a very subtle indicator of fatigue, the two processes varying directly with one another; and with alcohol the effect was in the same direction, whether the tests were done separately or together; therefore in so far as it might be possible to form *a priori* views about the question it would be expected that the effect of opium, which is to improve each test done separately would be analogous. Instead of this, however, the effect is much more complicated. In some cases the dotting is improved and the reproduction of words is deteriorated, in others the words improve and the dotting deteriorates.

There are eight cases of the double process with a dosage of 40 minims. A slight deviation in method was made in the case

[1] *Brit. J. Psychol.*, 1916, 8, 337.

[2] The subject (M. S.) had had much experience of the combined test under normal and fatigue conditions before doing it with alcohol.

[3] It will be noticed that the normal number of errors in dotting for the double process is fewer than when the dotting was done alone. This does not quite represent the position. The term to which this experiment belongs was one when all experiments were generally very well done; the normal average for this period for dotting done alone is 32. Sometimes for weeks together the dotting was very good; at other times for weeks it was poor.

of these experiments as only 3 metres of tape was used, and the list of words was read through once.

No.	Date.	Drug taken at	Errors immediately before taking the Drug. Dotting and Words.		Errors after taking the Drug. Dotting and Words.			hr.	min.
1.	Nov. 5, 1915	2.55 p.m.	33	20	43	11	interval	1	35
2.	May 10 ,,	2.45 p.m.	26	16	20	19	,,	2	
3.	May 23 ,,	4 p.m.	23	17	26	18	,,	2	30
4.	June 7 ,,	2.30 p.m.	23	15	21	21	,,	1	55
5.	June 14 ,,	3 p.m.	29	14	18	12½	,,	2	15
6.	March 20, 1917	4.45 p.m.	56	11	44	12	,,	1	35
7.	March 22 ,,	4.40 p.m.	47	21	71	16	,,	1	55
8.	March 25 ,,	4.55 p.m.	36	18	86	23	,,	1	40

It will be noticed that in two cases, Nos. 1 and 7, the dotting is deteriorated but the words are improved; in Nos. 2, 4, and 6 the dotting is improved very slightly but the words are deteriorated; in Nos. 3 and 8 both processes are deteriorated, and in No. 5 both are improved. On May 18, 1915, the two processes were done separately to see if the subject was ceasing to be affected by the drug, with the usual result, viz. considerable improvement in both tests, 40 errors in dotting being followed by 25 after the drug, and 16 in words before followed by 6 after.

With a dosage of 60 minims there are thirteen experiments, and one of 90 minims giving similar results.

No.	Date.	Drug taken at	Errors immediately before taking the Drug. Dotting and Words.		Errors after taking the Drug. Dotting and Words.			hr.	min.
1.	May 11, 1915	3.5 p.m.	28	11	37	8	interval	3	
2.	May 13 ,,	3.5 p.m.	25	16	19	11	,,	3	
3.	June 1 ,,	2.30 p.m.	28	12	36	16	,,	2	
4.	Nov. 10 ,,	3 p.m.	40	12	48	7	,,	2	
5.	Nov. 11 ,,	3 p.m.	27	11	31	11	,,	3	
6.	Nov. 17 ,,	3.35 p.m.	49	9½	50	18	,,	2	
7.	Nov. 18 ,,	3 p.m.	43	12	30	24	,,	2	
8.	Nov. 29 ,,	3 p.m.	49	10	38	15	,,	2	
9.	April 20, 1917	3.55 p.m.	35	4½	32	5	,,	1	45
10.	April 24 ,,	3 p.m.	38	12	33	1	,,	2	15
11.	May 1 ,,	3.5 p.m.	58	23	57	16	,,	2	
12.	May 5 ,,	4 p.m.	48	17	47	14	,,	2	
13.	May 7 ,,	4 p.m.	35	5	41	2	,,	2	30
14.	May 15 ,,	6.18 p.m. (90 minims.)	45	15	126	13	,,	2	15

It will be noticed that in Nos. 1, 4, 13, and 14 the dotting errors are increased, and the errors in words decreased; in Nos. 7, 8, and 9 the position is reversed; in Nos. 2, 10, 11, and 12 both processes are improved, but one process but slightly; in Nos. 3 and 6 both are deteriorated, while in No. 5 the dotting is slightly worse but the words unaltered. It will be noticed that in some cases the effect is very slight.

Subjectively, too, the difference before and after the drug is marked; it is, under no circumstances, easy to do the two tasks, i.e. dotting and listening to and realizing the relationship between words, together, but normally there seemed no particular tendency

to neglect one in favour of the other. After opium, however, the attention was always 'on the wing' in an irritating oscillation between one or other task, and it was quite possible for several sequences of words to pass by apparently unnoticed while interesting oneself in the dotting, or for a series of wild shots to be aimed at the dotting paper while attending to the words. Added to this was the dim awareness that the two tasks ought to be done together, but that this was not possible; it was as if one had work to do for which one felt quite incompetent; the total experience was thus far from pleasant. To do the two tests separately after having done them together was a delightful change. The amnesia for certain sequences of words while attending to the dotting was so great that, on being told during the reproduction what the words were, one often failed to recognize that they had ever been heard in that connexion.

Opium, as far as these experiments go, seems to have marked effects on the power of distributing attention; it improves the process of attention to an operation which can be readily dealt with as a unity by the subject, but it interferes with the power to distribute attention over more than one operation. It is perhaps this latter characteristic which is manifested in the well-known effects on people addicted to its use.

V. Perception of Ambiguous Figures as affected by Alcohol and other Drugs, Tea, and Fatigue.

In papers published by one of us many years ago,[1] it was argued that fatigue, alcohol, chloroform, strychnine, and some other drugs produce their most distinct and immediate results upon the working of the nervous system through acting upon the synapses or junctions of neurones.

Evidence in support of this view, as regards the local fatigue immediately resulting from continued activity of a nervous path or cerebral arc, was found in the study of those phenomena of conflict and reciprocal inhibition which are familiar to workers in psychological laboratories under the names of fluctuations of the attention and binocular rivalry.

It was obvious that if these arguments were well founded, they should find further support in the study of the effects of the drugs upon these oscillatory phenomena. Of all these the alternating appearances of ambiguous figures seemed best suited for such study, and especially the 'windmill illusion'. This illusion consists in the appearance of a periodic reversal of direction of rotation when the arms of a windmill are regarded from such a position that the line of vision falls obliquely to the plane of their movement.

This illusion is a more profitable object of study than other phenomena of fluctuating perception, because it can be partially controlled by the use of binocular vision, and because the alternating phases are perfectly definite and distinct, whereas in other phenomena of this order the distinct phases are apt to be separated by

[1] McDougall, 'Physiological Factors of the Attention Process', *Mind*, 1906, N.S. 15, 340. 'Conditions of Fatigue in the Nervous System', *Brain*, 1909, 32, 256.

periods of uncertainty and confusion, e. g. ambiguous figures drawn on the flat to represent solid figures (such as the cube or the staircase figures) are apt to be seen at moments as two-dimensional only.

For the purposes of this study a laboratory windmill, the arms of which consisted of four brass rods 18 inches in length, was driven at a constant rate of three revolutions per second by a water motor. In the majority of experiments the object observed was the shadow of these rotating arms projected obliquely upon a smooth white wall by a single lamp. This arrangement has the advantage that, although the shadow windmill always appears to be rotating definitely in one or other of two planes oblique to the wall on which it is projected, there is nothing to lead the observer to regard the apparent motion in one plane as more real than that in the other, the actual windmill being hidden from his view.

It was shown in the before-mentioned studies that the observer has a certain power of favouring one or other phase of such alternating phenomena by an effort of attention, and can, therefore, by such effort either retard or accelerate the rate of alternation. For the purpose of the present study, the plan was adopted of holding fast each phase for the longest possible period: i. e. the observer set his will to hold each phase, until the change of phase occurred in spite of his effort; and then directed his effort to hold fast the new phase. Under these conditions the duration of the phases and the rate of their alternation is more regular, and after a little practice becomes very constant for each observer; though showing remarkably wide differences in different observers.[1]

In one of us (McD.) the rate of alternation in his normal condition was 10–12 per minute; in the other (M. S.) it was about 6 or 7 per minute; and in a third subject it was about 2·5 per minute.[2]

(*a*) *Alcohol.*

The effect of a dose of alcohol upon this alternation of phases was in all cases a marked retardation of rate of alternation. The alcohol was taken in the form of absolute alcohol mixed with three times its bulk of water. The smallest dose was 10 c.c. of absolute alcohol. Within half an hour of its administration it had reduced the rate of alternation by nearly 50 per cent.[3] No other test yet devised will in our experience reveal any effect of so small a dose of alcohol in this subject (McD.), nor was any effect subjectively appreciable. Many series of experiments were made with doses ranging from 10 c.c. to 50 c.c. absolute alcohol. It is not possible to express the results in any numerical average; but it may be said in general terms that

[1] These differences are so large and constant that they seem to be the expression of very fundamental differences of nervous constitution, and are deserving of further study.

[2] Although the windmill illusion is best suited for such work, observations were made also upon the alternation of phases of the ambiguous cube figure (drawn on the flat) under the various conditions described below. The rate of alternations of this figure ran closely parallel to those of the windmill under all conditions.

[3] It is unfortunate that in these experiments no special attention was paid to the question of the condition of the stomach as regards food, but as they were made about noon or in the afternoon, and only a very light lunch was taken, the stomach was no doubt almost empty.

in every case the drug produced a large reduction of the rate of alternation of phases (and corresponding increase of duration of the successive phases): and that the extent of the changes were roughly proportional to the quantity of the drug taken. In many cases the observations were repeated at short intervals before and after taking the drug; and the nature of the change may best be illustrated by reporting one such series of observations which is typical of all.

January 26, 1914. Observer, McD.

2.50 p.m.	20 phases in 2 minutes
2.55 p.m.	20 ,, ,,
3 p.m.	50 c.c. Absolute Alcohol (with water) taken
3.5 p.m.	14 phases in 2 minutes
3.12 p.m.	5 phases in 2 minutes
3.17 p.m.	5 ,, ,,
3.34 p.m.	4 ,, ,,
3.45 p.m.	4 ,, ,,
4 p.m.	3 ,, ,,
4.5 p.m.	2 ,, ,,
4.17 p.m.	5 ,, ,,
4.32 p.m.	6 ,, ,,
4.42 p.m.	11 ,, ,,
5 p.m.	15 ,, ,,

From this table it appears that the effect of the alcohol gradually reached its maximum about 60 minutes after the drug passed into the stomach; after this period began to decline; and after another hour had almost passed away.

The corresponding and even more marked effect of alcohol upon the second subject (M. S.) may be illustrated by the following experiment:

The normal rate of alternation of phases is 6 or 7 per minute. She took 30 c.c. absolute alcohol. Fifteen minutes after the taking of this dose, the windmill maintained its apparent direction of rotation for 4 minutes without change; 30 minutes later it gave an initial phase lasting 35 seconds.

(*b*) *Chloroform.*

The effect of chloroform inhalation may be illustrated in a similar manner by citation of a series typical of several.

February 2, 1914. McD.

3.55 p.m.	11 phases in 60 seconds
3.57 p.m.	Four deep breaths of vapour from a piece of lint sprinkled with chloroform and held loosely over the face. This was sufficient to cause a slight giddiness, with buzzing in the ears, and a sense of swelling of the face;
immediately thereafter	2 phases in 60 seconds
4 p.m.	4 ,, ,,
4.2 p.m	7 ,, ,,
(feeling almost normal)	
4.3 p.m.	9 ,, ,,
4.5 p.m.	10 ,, ,,
4.8 p.m.	12 ,, ,,

From this table it appears that chloroform for the dosage used in this experiment produces an effect like that of alcohol; but that,

as might be expected, the effect reaches its maximum within 1 or 2 minutes, and thereafter rapidly declines and passes away within 10 minutes.

In the second subject (M. S.), whose normal rate of alternation was 7 per minute, similar inhalation of chloroform immediately reduced the rate to five phases in 2 minutes.

In a third subject whose normal rate was 5 phases in 2 minutes, similar inhalation prolonged the first phase of the observation immediately succeeding it to 170 seconds and the second phase to 85 seconds.

(c) Strychnine.

The effects of strychnine on this test were studied by one observer only (McD.) who swallowed the drug on an almost empty stomach in the form of tabloids of strychnine nitrate.

They may be illustrated by the following table which shows also the intercurrent effect of alcohol:

February 26, 1914.

2.15 p.m.	11 phases in 1 minute
2.20 p.m.	10 ,, ,,
2.30 p.m.	$\frac{2}{15}$ gr. strychnine nitrate taken
3.15 p.m.	$\frac{1}{15}$ gr. ,, ,, ,,
3.40 p.m.	16 phases in 1 minute
4 p.m.	17 ,, ,,
4.15 p.m.	19 ,, ,,
4.30 p.m.	30 ,, ,,
4.35 p.m.	25 ,, ,,
4.45 p.m.	30 c.c. absolute alcohol taken in water
5.15 p.m.	7 phases in 1 minute
5.20 p.m.	8 ,, ,,
5.45 p.m.	12 ,, ,,
6.45 p.m.	15 ,, ,,
7.15 p.m.	18 ,, ,,
7.20 p.m.	18 ,, ,,

This series shows a marked effect of strychnine in accelerating the alternation of phases, an effect which reaches its maximum rather more than two hours after the passage of the drug into the stomach, and declines so slowly that three hours later it is still very marked.

The same series illustrates also the antagonism between strychnine and alcohol. The latter drug was taken at the time when the effect of the former was beginning to decline. Within half an hour it had overcome the effect of the strychnine, but was prevented by the latter from producing its full effect: half an hour later the strychnine began to predominate. After another hour the effect of the alcohol was still manifest, but after the further lapse of 45 minutes the alcohol effect seemed (in accordance with previous observations) to have passed away, leaving the declining strychnine effect to manifest itself unopposed.

A similar series of observations extending over eight hours was made on February 13, showing a similar effect of strychnine ($\frac{1}{5}$ gr.), namely, a gradually increasing acceleration of rate of alternation, reaching its maximum (33 phases per minute) about $2\frac{1}{2}$ hours after the passage of the drug into the stomach. In this case the declining curve of the strychnine effect was interrupted by the inhalation of chloroform, which immediately reduced the rate of alternation from 24 to 3 per minute.

In a third similar series $\frac{4}{15}$ gr. of strychnine nitrate produced a gradual acceleration, reaching 25 phases per minute after three hours, at which time the observations were discontinued.

From these experiments it appears that in this subject strychnine acts in a manner directly the opposite of alcohol and chloroform in respect of the 'windmill' illusion; and that its effect is counteracted by them more or less completely, according to the dosage.

(*d*) *Tea.*

The same subject tested the effect of tea by taking two large cups of very strong tea (the infusion of seven large teaspoonfuls). Within half an hour of the drinking of this brew, the rate of alternation rose to 27 per minute, declining to 17 per minute after a second half-hour.

(*e*) *Opium.*

In the same subject 80 minims of tincture of opium, taken by the mouth in a cup of weak tea at 1.30 p.m. was followed by acceleration from 10 phases per minute at 1.30 p.m. to 18 at 3 p.m., 20 at 3.10 p.m., and 22 at 3.45 p.m. On another occasion 30 minims of tincture of opium accelerated the alternation from 10 to 16 per minute after 90 minutes.

From this it appeared that opium exerts an effect the opposite of alcohol, but slower in onset and decline. These opposite effects are illustrated in the following series:

March 30. Subject, McD.

Noon	12 phases in 60 seconds.	
12.15 p.m.	30 c.c. Absolute Alcohol plus 50 minims Tincture of Opium by the mouth	
12.30 p.m.	9 phases in 60 seconds	
12.45 p.m.	9	,, ,,
1 p.m.	8	,, ,,
1.15 p.m.	7	,, ,,
2.45 p.m.	14	,, ,,
3.15 p.m.	18	,, ,,
3.45 p.m.	17	,, ,,
4.30 p.m.	16	,, ,,

This series illustrates the antagonism of opium and alcohol; before the alcohol can attain its maximum effect, it is partially counteracted by the opium, which, when after two hours the alcohol effect has declined, asserts itself in a reversal of the effect, attaining its maximum at the end of the third hour, and declining appreciably during the fourth hour.

In the other subject (M. S.) the windmill illusion was observed on five occasions after taking tincture of opium in doses varying from 40 to 90 minims. In all cases the rate of alternation of phases was markedly increased, the maximal observed rate ranging from 18 to 33 per second, according to the quantity of the drug taken.

(*f*) *Potassium Bromide.*

This observation is perhaps worth noting as some guarantee that the effect of drugs on the windmill illusion described above, is not due to expectation or auto-suggestion. It was anticipated

that potassium bromide would retard the alternations of phases: but no appreciable effect, either of retardation or acceleration was observed, even after as large a dose as 80 grains of potassium bromide.

(*g*) *Fatigue.*

In the paper referred to above[1] it was shown that when, by the use of binocular vision at close quarters, the apparent direction of movement of the windmill is maintained in one phase for an abnormally long period, this is followed by a prolonged phase of the opposite sign; that is to say, the local fatigue resulting from continued activity of a cerebral path (having its seat in all probability at the synaptic junction of the path) affects the phenomenon in the same way as alcohol and chloroform.

Miss Smith's studies of fatigue[2] have shown that deprivation of sleep results in a period of increased efficiency, followed by a longer period of diminished and slowly-recovering efficiency, in the concentration of attention. This initial period of increased efficiency suggests that the nervous system temporarily maintains its efficiency against oncoming exhaustion by generating some chemical substance which facilitates its operations. It is of interest, therefore, to find that, during this period of increased efficiency or facilitation of effort, the windmill illusion shows a well-marked acceleration of phases, i.e., it is affected in the same way as by strychnine, opium, and tea. This appeared clearly in two of her 'fatigue cycles' in which daily observations of the windmill illusion were made. After three nights of much shortened sleep, the rate of alternations rose from 7 a minute to 35 a minute, and only slowly returned to the normal rate in the course of a fortnight.

Summary.

The observations described above are so few and made by so small a number of subjects that they cannot be made the basis of any confident generalizations. The war has interrupted the prosecution of this method and has indefinitely postponed the prospect of their resumption by us, and we now publish them as a suggestion and clue to other workers. They suffice only to suggest that in the observation of the windmill illusion we have a very delicate method of manifesting in a quantitative manner such changes of the condition of the brain as result from fatigue and the action of drugs, such as alcohol, chloroform, strychnine, opium, and tea. They suggest that these drugs (and probably others which markedly affect the nervous system) fall into two groups of opposite effect; that those of one group (alcohol and chloroform) act chiefly and immediately by raising the resistances of the synapses in all parts of the brain, while those of the other group (strychnine, opium, and tea) produce their opposite effects by lowering the synaptic resistances; further, that the nervous system, when subjected to strain, maintains its efficiency for a time by producing a substance similar in its effects to the drugs of the second group, a substance which perhaps, like them, is of the nature of a poisonous alkaloid.

[1] *Mind*, 1901, N.S., 10.

[2] *Brit. J. Psychol.*, 1916, 8, part III.

These suggestions as to the nature of the effect of drugs and fatigue on the nervous system are borne out by the prolonged observations of Miss Smith of their effects upon the process of 'dotting' and of memorizing (related words test), in both of which efficiency depends upon a general concentration of attention. For the general changes in efficiency revealed by these tests under fatigue, alcohol, and opium, are all such as would seem to be satisfactorily explained by the adoption of the hypothesis suggested above.

VI. Subjective Effects of the Drugs.

(*a*) *Alcohol.*

These subjective descriptions are all taken from the period during which the subject was in ignorance as to which drug, if any, had been taken; the statements did not all occur on any one occasion, but are extracts from many. As the general effect of a drug on consciousness is not infrequently a state for which there is no specific word, it is necessary to fall back on metaphor.

'Shortly after taking *X*, I felt very dizzy and heavy, had great difficulty in making myself begin anything, found it difficult to make an effort—feeling as if the back of my head were not there—light headed—general state akin to that in passive attention—felt as if I were a diagram, i.e. attenuated—thought the dotting was very good and words easy—tendency to perseveration, although physically disinclined to begin anything.'[1]

During the period of ignorance this drug was distinguished as the 'dizzy' one, from its most obvious effect.

(*b*) *Opium.*

'My limbs felt as if recovering from cramp. I felt "nervy", as if about to undergo an ordeal, but without the attendant organic sensations, a feeling of excitement; I felt tired; tingling sensation, the pen felt very small so that the dotting seemed neat and dainty,[2] the time of the experiment seemed short; my head felt as if it were beginning to ache but in a way too undefined to be actual pain; a feeling of restless activity not unlike the first stage of excessive fatigue followed by a feeling of drowsiness combined with mental alertness; anticipatory feelings without specific object, i.e. a feeling as if something were going to happen, giving a vague fear of the unknown, sometimes pleasurable, sometimes painful, determined by factors I could not isolate.' This latter state is not exclusive to opium most people being aware of it occasionally under apparently normal conditions, but it occurred so frequently in the records which proved to be opium days, that it seems to be a direct drug effect.

Although most tasks were improved by opium, yet the success was always somewhat tremulous, a success which appeared to be

[1] See 'Alcohol' by Members of the Advisory Committee to the Central Control Board (Liquor Traffic), ch. 3, p. 30.

[2] An object falling, however, so as to be seen indirectly appeared unnaturally large.

uncontrollable and therefore surprising; familiar though the subject became with these opium effects, yet surprise was always experienced at the resulting improvements. These subjective effects all became less marked in time. A feeling of drowsiness sometimes lasted several hours afterwards.

VII. Some Practical Applications to the Study of Alcohol.

Having found that alcohol has measurable effects, and that the dotting test was a sensitive indicator of these effects, it was decided, at the suggestion of Lord d'Abernon (chairman of the Central Control Board of the Liquor Traffic), to apply this knowledge to the following problems:

(1) To find out what was the effect, if any, of the degree of the dilution of the alcohol.

(2) To compare the effects of alcohol taken immediately after a meal with its effects several hours after a meal.

(*a*) *The Effect of the Degree of Dilution.*

In this investigation only the dotting experiment was used and the tests could only be undertaken by one of us (M.S.).[1] The experiments were done after the lapse of about two years from most of the previous drug experiments. In a preliminary series of experiments with different degrees of dilution it was found that little difference was revealed between the concentrated and diluted solutions, but as these were done within an hour of a meal the reason was not far to seek, as will be seen by reference to the next section. In the final experiments undertaken some months after, an interval of at least $2\frac{1}{2}$ hours from a meal had elapsed before beginning the experiments.

Two degrees of dilution were used:—

(1) 20 c.c. absolute alcohol diluted to 60 c.c. with water.
(2) 20 c.c. „ „ 200 c.c. „

The two sets were done for the most part on alternate days but no definite rule was observed, and the experiments extended over a period of several weeks.

It had been found in previous investigations that the maximal effect of such doses of alcohol as were used in these experiments occurred about 30 minutes after taking the dose; this interval was therefore chosen on those occasions when records every few minutes could not be obtained.

The method was as follows:

After an interval of at least $2\frac{1}{2}$ hours from a meal the usual length of tape was dotted, then the solution of alcohol, diluted or concentrated, was taken, and after an interval of 30 minutes another strip of the tape was dotted.

[1] The other tests which had proved to be refined enough for the purpose, i.e. the lists of related words and the windmill, could not be used owing to war time conditions; only one of the writers was available for the experiments, and that at less certain times, and there was no laboratory accommodation such as is required to operate the windmill.

Details of the Experiments.

A. Concentrated Solution.

Date.	Interval from preceding Meal.	Errors before taking the Drug.	Errors 30 Minutes after taking the Drug.
June 2, 1919	$2\frac{1}{2}$ hrs.	56	170
June 10, 1919	$2\frac{1}{2}$ hrs.	35	73
June 12, 1919	4 hrs.	45	128
June 16, 1919	$2\frac{1}{2}$ hrs.	42	135
June 18, 1919	$2\frac{1}{2}$ hrs.	46	65
June 19, 1919	$3\frac{3}{4}$ hrs.	33	101
		Mean = 43	Mean = 112

160 per cent. increase in the number of errors.

B. Diluted Solution.

June 7, 1919	$3\frac{3}{4}$ hrs.	36	29
June 9, 1919	$2\frac{1}{2}$ hrs.	46	70
June 15, 1919	$2\frac{3}{4}$ hrs.	47	64
June 20, 1919	4 hrs.	46	61
June 23, 1919	$3\frac{3}{4}$ hrs.	52	61
June 24, 1919	$2\frac{1}{4}$ hrs.	42	46
		Mean = 45	Mean = 55

22 per cent. increase in the number of errors.

The average number of errors before taking the alcohol of the more concentrated solution was 43; for almost 25 minutes after taking the dose there are only normal variations, but 30 minutes after, the errors rise to an average of 112, an increase of about 160 per cent., after which there is a gradual return to normal. In the case of the experiments with the weaker solution the average number of errors before taking the alcohol is 45; 30 minutes after the errors rise to 55, an increase of 22 per cent. In one case with the weaker solution there is a slight improvement, and in the other cases the increase is very little greater than might have occurred as a normal variation; the concentrated solution, on the other hand, in every case gives a variation after alcohol much greater than could have been due to a normal variation.

Thus the weaker the solution, the less marked is the effect. Subjectively the same experience occurred; both the solutions were noticeable subjectively, but the concentrated one was much more so.

(*b*) *The Effect of a preceding Meal.*

Tests.—The tests used were (i) the dotting; (ii) lists of related words; (iii) a few experiments with the aesthesiometer.

Dosage.—30 c.c. absolute alcohol diluted to 60 c.c. with water.

Time of day.—This was kept constant for both sets, viz. from 5.45 p.m., except in one case, that on April 29th which was done before breakfast.

Results.

(i) *The Effect on the Dotting.*

Date.	No. of Hours from a Meal.	No. of Errors immediately before taking the Drug. Dotting.	Words.	Errors 30 Mins. after taking the Drug. Dotting.	Words.
March 16, 1919	5	26		103	
March 26, 1919	2½	32	4½	92	19½
April 2, 1919	2	39	9	93	21
April 29, 1919	11	39		90	
April 30, 1919	4½	44	6	96	19
May 4, 1919	4¼	50	6½	85	12
May 11, 1919	4⅓	36		56	
May 21, 1919	4¼	34	7½	56	16
June 2, 1919	4	45	6	79	15
		Mean = 38	Mean = 6.6	Mean = 83	Mean = 17

For the nine experiments done several hours after a meal the average number of errors in dotting before taking the drug was 38. Within 30 minutes of taking the drug the errors rise to 83, an increase of 118 per cent. Subjectively, the effects of dizziness and a feeling of constriction of the muscles of the face showed themselves within 15 minutes and increased in intensity for 30 minutes.

Date.	No. of Errors before taking the Drug. Dotting	Words.	No. of Errors 30 Mins. after taking the Drug. Dotting	Words.
Feb. 18, 1919	32		39	
Feb. 21, 1919	33		39	
May 25, 1919	39		35	
June 5, 1919	40	7½	44	6½
June 9, 1919	32		28	
June 11, 1919	27	8½	29	13½
	Mean = 34	Mean = 8	Mean = 36	Mean = 10

For the six experiments done immediately after a meal[1] the average number of errors before taking the drug is 34, and the average is only 36 errors 30 minutes after taking the drug, barely 6 per cent. increase; this result is in marked contrast to the previous set. Subjectively, too, the symptoms were so slight as to escape notice unless attention was given to them.

(ii) *The Effect on the Lists of Words.*[2]—The average number of errors before taking alcohol for those days when no meal had been taken for several hours was 7; 30 minutes after the drug the errors rose to an average of 17. For the two experiments done immediately after a meal the average number of errors was 8; 30 minutes after, 10.

(iii) *The Effect on the Aesthesiometer.*[3]—The distance taken as the standard was 25 cm. 4½ hours after a meal the average number of errors was 24 per cent.; after alcohol the errors rose to 90 per cent. When, however, the test was done immediately after a meal, the errors varied from 18 per cent. before to 27 per cent. after, a quite small deviation.

Summary.—Alcohol to the amount of 30 c.c. taken with a meal has very little effect as far as these tests are concerned and the

[1] For the purpose of these experiments the meal taken was a light one, usually tea, bread and butter, and an egg.

[2] Lists of words could not be done every time as a collaborator was needed.

[3] See McDougall, *Cambridge Anthropological Expedition to Torres Straits*, vol. ii, p. 189; Burt, *Brit. J. Psychol.*, 1909, 3, 115, for details of procedure.

subjective effects are very slight. But when alcohol is taken several hours after a meal it has marked effects and shows itself subjectively within ten minutes of taking the drug and objectively involves a loss of muscular control, failure of memory, and deterioration in skin sensitivity.

VIII. General Summary.

Within the limits of these studies we have shown:

(*a*) That there are tests (and these of the higher processes) which definitely and unambiguously enable us to study objectively the effects of drugs, and that these tests are of practical application.

(*b*) That the subjective effects by no means are a safe criterion of the objective result.

(*c*) That the drugs investigated fall into two distinct groups antagonistic to each other, viz. alcohol, chloroform, and the second phase of fatigue, as against strychnine, opium, tea, the immediate effects of fatigue, and alcohol acting during certain fatigued states.

(*d*) That fatigue caused by loss of sleep has an action resembling that of drugs.[1]

[1] Cf. Rivers, *Influence of Alcohol, &c., on Fatigue*, p. 119.

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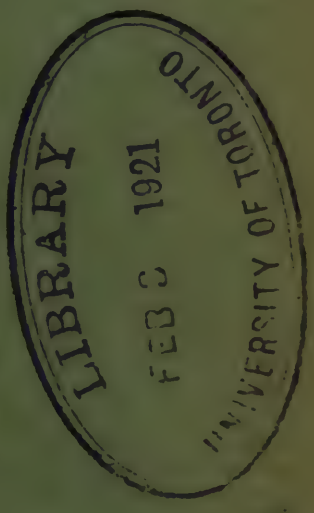
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STUDIES IN WOUND INFECTIONS





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INTRODUCTION

In the studies here presented an account is given of work done during the twelve months beginning July, 1916, in the Inoculation Department of St. Mary's Hospital, where at that time the bacteriological staff of the Medical Research Committee were temporarily accommodated. As part of the contributions which the Committee were then making to the scientific study of wound-infections and wound-treatment, both with the Forces and at home, it had been arranged early in 1916 that 25 beds in this department of St. Mary's Hospital should be set aside for the special study and treatment of suitable cases sent to the wards direct from No. 13 General Hospital, Boulogne, where Sir Almroth Wright, at that time Director of the Committee's Bacteriological Department, was serving as Consultant to the Forces. It will be seen from the Report that the use of these research wards for the treatment of this group of cases of severe septic wounds allowed the intimate association of laboratory with clinical work, by which treatment in all its stages could be guided and the results scientifically observed. The authors of the Report, to whom the researches now described were entrusted, had entire clinical charge of the patients. This work took its place among contemporary studies being made elsewhere, and among these it had at the time immediate complementary value. It has never been possible until now, however, for these results to be published in full. Near the close of the war Captain Douglas's health broke down for a time, and his colleagues were long absent abroad upon military service. It has only been recently possible for them to collaborate in bringing together their accumulated results.

The Medical Research Council would not have it thought that the authors now bring forward this work at this date as offering anything like a complete summary of the knowledge of wound-infections gained during the war. The present report should be regarded as taking its place among other studies in the same

(1737) Pa 1263 Wt. 11827 296 948 1500 12 21 (9 17 8)

subject which were made at that time. Though the publication has been unavoidably delayed, the Council are confident that the observations here recorded, of which only some having immediate practical value have been hitherto published, ought now to be made available together in the present form and will be widely welcomed.

30 *October*, 1920.

Medical Research Council,
15 Buckingham Street,
Strand, London, W.C. 2.

STUDIES IN WOUND INFECTIONS

BY

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CONTENTS

PART I

PAGE

A. Nature of the Cases Treated, Methods of Treatment Employed, and some Conclusions arrived at 7

(1) Nature of the cases treated 7

(2) The dressing of the wounds 7

(3) Skin grafting 8

(4) Methods adopted for promoting the growth of epithelium over the wounds 8

(5) Measures adopted to alleviate the pain of dressing the wounds . . 9

(6) The employment of perforated celluloid sheets as splinting material . 11

(7) The application of massage as a means of hastening the healing of indolent wounds 11

(8) Vaccine therapy 12

(9) The advantage of using an autogenous vaccine in certain cases . . 13

(10) The operation of secondary suture 16

(11) The persistence of living bacteria in scar tissue and sequestra . . 17

B. General Observations on the Bacteria found in the Wounds . . 18

(1) The faecal origin of wound infections 18

(2) The importance of *Streptococcus pyogenes* 18

(3) The recrudescence of bacterial infections after operations . . . 19

PART II

A detailed Account of the Characteristics of the various Bacteria isolated from the Wounds 20

(1) Introduction—and routine procedure employed in studying the bacterial flora of the wounds 20

(2) The anaerobic bacteria 21

(*a*) *General considerations* 21

(*b*) *Classification* 23

(*c*) *The non-sporing anaerobic bacteria* 24

(*d*) *The non-putrefactive group of spore-bearing anaerobic bacilli* . . 25

(*e*) *The putrefactive group of spore-bearing anaerobic bacilli* . . . 37

(*f*) *Bacteria that cannot be placed in any of these groups* . . . 47

(3) The Streptococci 49

(*a*) *Methods of differentiation* 49

(*b*) *Some general considerations with regard to Streptococci* . . . 50

(*c*) *Classification of the strains of Streptococci isolated from 50 cases taken at random* 51

(*d*) *The characters of the various types of Streptococci* 51

PAGE

(4) The diphtheroid bacilli 61
(a) *Definition* 61
(b) *Number of wounds infected* 61
(c) *Possible sources of infection* 61
(d) *Methods of isolation and differentiation* 62
(e) *Characters of 61 strains of diphtheroid bacilli* 62
(f) *Incidence of the different types of diphtheroid bacilli* . . . 67
(g) *Significance of infection with diphtheroid bacilli* 68

(5) *B. proteus* 69
(a) *Number of wounds infected and persistence of the infection* . . 69
(b) *The isolation and general characters of* B. proteus . . . 69
(c) *Serological races of* B. proteus 71

(6) *B. pyocyaneus* 71
(a) *Number of wounds infected—and variation of the strains as regards chromogenic power* 71
(b) *Pathogenicity* 72

(7) *Staphylococci* 72

(8) Coliform bacilli 72

PART III

EXPERIMENTAL WORK BEARING UPON THE ACTION OF CERTAIN ANTISEPTICS IN INFECTED WOUNDS 73

(1) Introductory 73

(2) Methods of experimentation 73

(3) Experiments bearing upon the ability of antiseptic solutions to kill bacteria in the discharges from an infected wound 74

(4) Experiments carried out with a view to showing why the antiseptics in common use fail to sterilize infected wounds 77
The destruction of antiseptic potency in wounds 77

(5) Experiments *in vitro* bearing upon the bactericidal efficacy of antiseptics in the presence of serum 80
(a) *Inhibitory effect exerted by antiseptics on the growth of bacteria in a serous medium* 83
(b) *Stimulant effect of antiseptics on the growth of bacteria in serum* . 84

(6) Experiments which serve to demonstrate the fixation of certain antiseptic dye-stuffs by the fabrics commonly employed in the dressing of wounds 90

(7) Experiments made to ascertain whether certain antiseptic solutions were capable of penetrating into—and killing the microbes contained within—small diverticula such as exist in all septic wounds . . 91

(8) Experiments dealing with the ability of certain antiseptics to penetrate the walls of the wound 95

(9) Investigation of certain changes brought about in the tissue and blood cells as the result of the introduction of antiseptics into the wound 98
(a) *The action of antiseptics upon the fixed tissues* 98
(b) *The destructive effect of antiseptics on leucocytes* 99

(10) The effect produced by certain antiseptics upon the exudation of fluid into the wound—and upon the properties of that exudate . . 101
(a) *The rate of exudation* 101
(b) *The albumen content of the exudates* 107
(c) *The number of leucocytes in the exudates* 107
(d) *The antitryptic value of the exudates* 108

(11) Experiments bearing upon the employment of hypertonic salt solutions in the treatment of infected wounds 112
(a) *The rate of exudation* 112
(b) *The albumen content of the exudate induced by salt solution* . . 116
(c) *The number of leucocytes contained in the exudate* 116
(d) *The effect of hypertonic salt solution upon the antitryptic value of the exudate* 117

(12) Summary and discussion of results 117

APPENDIX. Clinical Account of the 61 cases, with Bacteriological Notes . 122

PART I

A. NATURE OF THE CASES TREATED, METHODS OF TREATMENT EMPLOYED, AND SOME CONCLUSIONS ARRIVED AT

(1) Nature of the cases treated.

The clinical reports given in the Appendix will serve to show that the infections of the cases investigated by the authors were, on the average, rather more than usually severe. Many of the patients had, in the earlier stages, been affected with gas gangrene, injuries to the bones, &c. The period which elapsed after the infliction of the wound to the time when the patients were admitted to the wards of the Inoculation Department varied considerably, the average being eighteen days, but in some of the cases only four days had elapsed, and in many the investigations were commenced within a week of the infliction of the wounds.

(2) The dressing of the wounds.

The routine method of treatment in the early stages of the wounds was the use of packs moistened in 5 per cent.—or, if this caused pain, in 3 per cent.—salt solution. No attempts were made to compare the results obtained by this treatment with those obtained by the use of the various antiseptic solutions, as it was considered that the beds available would not accommodate a sufficient number of cases for results to be obtained, and that in any case the conclusions drawn from such comparisons are very frequently fallacious.

The details of the method of dressing wounds in the early stages were as follows: after the surface had been washed over with some solution such as normal salt, or occasionally, on account of its mechanical cleaning properties, with a solution of hydrogen peroxide, either of which were applied by means of a spray producer,[1] the wound surface was covered over with many layers of butter cloth which had been soaked in 5 per cent. salt solution. The butter cloth was usually folded in four and made either into square pads or into rolls about 4 inches wide and some yards in length. Care was taken gently to pack the butter cloth into any cavities or recesses there might be in the wound surface. Coiled amongst the folds of butter cloth were one or more Carrel tubes. Over the wet mass of butter cloth sheets of oiled paper were placed, care being taken that the paper freely overlapped the wet dressings in every direction. Small holes were cut in the oiled paper and the open ends of the rubber tubes were brought through them. The whole dressing was then fixed in position by a bandage, the rubber tubes passing between the layers. If the oiled paper and bandages had been carefully applied no leakage of the salt solution took place. At intervals of

[1] A full description and illustration of the type of spray producer employed is given in the paper on 'Skin-grafting', by the present authors. See *Lancet*, 1917, ii. 5.

about four hours a quantity of salt solution could be supplied to the dressing in contact with the wound surface by fitting a syringe filled with 5 per cent. salt solution on to the open end of the rubber tube and injecting its contents, which were in this way evenly distributed through the numerous small openings in the walls of that portion of the tube which was coiled in the dressings. This procedure caused no disturbance to the patient, and was frequently carried out during the night without even awaking him. Such dressings were changed at first every twenty-four hours; later, when the wound was progressing favourably, every second day, and the intervals between the injections of salt solution into the dressing were proportionately prolonged.

As the result of this treatment the wounds cleaned very rapidly, any sloughs being rapidly digested away and any redness or inflammatory infiltration of the surrounding tissues disappearing. The numbers of bacteria seen in films made from the discharge from the wound decreased enormously, so that after the treatment had been carried out for several days it was often necessary to examine many fields of such films to ascertain the types of bacteria present. The surface of the wound became covered with small firm and quite painless granulations and the area began to decrease owing to the contraction of the newly-formed scar tissue. It was during this stage that many of the wounds could be partially or completely closed by secondary suture; this procedure will be discussed in more detail in a later section. After the wound surface had become covered with granulations normal saline was usually used in place of the 5 per cent. salt solution.

(3) Skin-grafting.

When the area of the wound had been somewhat reduced by the contraction of the newly-formed scar tissue and the epithelium had started to spread from the skin edge many of the wounds were skin-grafted. A method of grafting which had been employed in 1870 by an English surgeon, named Steele, was revived—with some modifications—and proved of the greatest value. Further, it was found possible to dispense with general anaesthesia for the operation by substituting the method of regional anaesthesia induced by nerve blocking. Some thirty-three transplantation operations of this kind were performed. The technique employed, and the results obtained, have been fully described in a paper by the present authors (vide *Lancet*, 1917, ii. 5).

(4) Methods adopted for promoting the growth of epithelium over the wounds.

To encourage the growth of the epithelium and to keep the granulating surface in a condition favourable for the spread of the epithelium from the edges the usual routine was to dress the wound with some astringent solution such as that commonly known as 'red lotion' or more frequently with a solution of either malachite or brilliant green.

That these aniline dyes had a favourable action on the growth

of epithelium was demonstrated on several occasions by dressing half of the wound with a solution of the dye and the other with a normal salt solution. The depth of the new epithelial edge was carefully measured by placing a sheet of clear celluloid over the wound and through it tracing the exact outline of the edge of the scar and the extreme edge of the new epithelium. Such measurements were made before the experiment was commenced and again after an interval of some days. The results obtained demonstrated conclusively that the new epithelium had spread considerably farther over the granulations on the half of the wound dressed with the solution of the dye than on the portion dressed with normal salt solution. For instance, half the surface of a granulating wound had a 1 per cent. solution of malachite green in spirit applied to it daily by means of a spray, the other half being carefully protected during the spraying process. The whole wound was then dressed with a pad of butter cloth moistened in normal salt solution over which was placed a sheet of oiled paper to keep the dressing moist. Before and after this treatment had been carried out for a week, careful tracings were made of the breadth of the new epithelium, and from them measurements were taken at corresponding positions of the epithelial border on the two halves of the wound. The following results were obtained :

Portion sprayed with dye.

	cm.	*cm.*
Before treatment	0·9	0·56
After one week's treatment	1·53	1·11
Increase =	0·63	0·55

Portion not sprayed.

	cm.	*cm.*
Before treatment	0·72	0·63
After one week's treatment	0·82	1·1
Increase =	0·1	0·47

The more usual method was to use a 1 per cent. watery solution of brilliant green which was applied once a day ; a piece of butter cloth several folds thick, cut to the size of the granulating surface, was soaked in the solution of the dye, and after being laid on the wound was covered with oiled paper sufficiently large freely to overlap the wet dressing in every direction.

(5) Measures adopted to alleviate the pain of dressing the wounds.

The next point to be discussed was one which very early in the course of the investigation was constantly brought before the notice of the authors, namely the pain inflicted on the patients during the process of dressing their wounds owing to the sticking of the dressings to the wound surface. This pain, especially in the early course of the wounds or after operations for the treatment of gas gangrene or amputations by the flapless method, was very severe, and all attempts to prevent or alleviate it by keeping the dressings wet were quite ineffective. A method was, however, devised, and the following note describing it appeared in the *Lancet*, September 23, 1916 :

' The problem of the alleviation of the pain caused by the removal of dressings which have become adherent to the wound is, at present, of more than usual

importance owing to the types of wounds from which so many of the soldiers are at present suffering.

'The special types referred to are those which either as a result of the primary injury, or of the operative measures necessitated by the onset of gas gangrene, have lost considerable areas of skin. In such cases the dressings are at times so painful as to act very detrimentally on the patients' general condition. Various methods were tried with the view of solving this problem, the principle of all being to place next the wound surface some non-absorbent material which, however, would allow free passage outwards of any discharges and inwards of any fluid that it was desired should reach the surface of the wound. After the trial of several materials, it was found that there were on the market sheets of perforated celluloid 0·15 millimetre in thickness, the perforations being 1·5 millimetres in diameter, and there being four perforations to each centimetre. This material, which was rather too stiff to be conveniently applied to the irregular surface of the wound, was found to become perfectly soft and pliable, and at the same time somewhat elastic, after it had been soaked in a 5 per cent. carbolic acid solution for a few hours. The carbolic acid solution having been washed away with sterile salt solution, the softened celluloid can be applied to the wound surface and falls at once into all the irregularities, and any suitable dressing can be applied over it. On redressing the wound it is found: (1) that the celluloid lifts off the surface of the wound without causing any pain; (2) that all the discharges from the wound have passed through the perforations, leaving the surface of the wound quite clean; and (3) that the celluloid has regained its original stiffness, thus making an accurately fitting splint, which tends to keep the wounded tissue in a complete state of rest. After the celluloid has been taken off the wound it is cleansed in tepid water and again softened and sterilized by placing it in the 5 per cent. carbolic acid solution. A convenient plan is to have two pieces of the celluloid for each wound, one of which is applied to the wounded area, the other being kept in a 5 per cent. solution of carbolic acid so as to be ready for use.

'This material can be obtained in varying thicknesses, the perforations being of the same size and number, and it has been found that the thicker sheets make an excellent material for the making of splints, especially for those cases for whom continuous or intermittent irrigation forms part of the treatment. For this purpose the sheets of perforated celluloid, having been first softened in 5 per cent. carbolic acid solution, are moulded to the injured limb. After the celluloid has again hardened it is attached to a metal framework such as may be readily made with the aluminium skeleton splinting supplied in the field fracture-box.

'It was at first thought that the wound granulations would tend to grow through the perforations, but this has occurred only in those cases in which the granulations tended to be oedematous, and in these the celluloid is not required, as ordinary gauze dressings showed no tendency to become adherent to the wound surface.

'The material was obtained from the British Xylonite Company, Hale End, Walthamstow, London.'

The perforated celluloid recommended in the above note answered perfectly in wounds the surfaces of which were moderately flat, but when there were marked irregularities, or, in the case of certain wounds such as those resulting from flapless amputations, the stiffness of the celluloid was a distinct disadvantage. Investigations were, therefore, continued and latterly a seemingly almost perfect material was evolved: this is manufactured by Messrs. Maw, Son & Sons, and is supplied by them under the name of 'Perforated Parex Protective'. It is very similar to oiled silk, closely perforated and possessing over and above its extreme suppleness the advantage that it can be sterilized by boiling. It is used in exactly the same manner as the perforated celluloid except that owing to its suppleness a fold can be tucked into any irregularity. These folds are, in the next stage of the dressing, filled in with a piece of the wet gauze or other material which may be used for dressing the wound.

When the wound treated in this way is redressed the removal of the gauze even from deep cavities causes practically no pain and the perforated material shows no tendency whatever to become adherent to the raw surface. Further, drainage has not in any way been interfered with but rather encouraged, the surface of the wound being found extraordinarily free from pus.

(6) The employment of perforated celluloid sheets as splinting material.

Slightly thicker perforated celluloid (e. g. $\frac{10}{1000}$ or $\frac{15}{1000}$ of an inch) was frequently employed for splint making, as it was found to provide an excellent flooring on which limbs could rest when immobilized in Thomas or other improvised skeleton splints. For this purpose the celluloid, after being thoroughly softened in 5 per cent. carbolic acid, was laid loosely, but without creases, across the skeleton frame, to the bars of which it was firmly secured by clips or thread. In the neighbourhood of open wounds on the dependent surface of the limb it was either interrupted or a sufficient window was cut and the edges turned over to avoid any chafing of the skin. The advantages of this procedure over the use of rubber or flannel slings seemed to the authors to be as follows :

(1) The celluloid in hardening took the shape of the limb, thus distributing its weight evenly, so ensuring comfort, immobility, and minimal risk of pressure sores. The avoidance of sores was also favoured by the free access of air to the skin through the perforations.

(2) Owing to the material being quite non-absorbent intermittent irrigation could easily be employed, the fluid escaping through the perforations. It was also very easily kept clean.

(3) The celluloid being almost transparent it permits the condition of the limb to be seen without its being in any way disturbed.

(7) The application of massage as a means of hastening the healing of indolent wounds.

In wounds where there had been an extensive loss of tissue as a result either of the actual injury or of operations undertaken for gas gangrene, the spread of the epithelium from the edges frequently became very slow during the later stages of treatment. On examination of such wounds it was found that immediately below the granulating surface there was a mass of dense scar tissue, the presence of which appeared to have the effect of strangling the lymph flow to such an extent as to be an important factor in bringing the healing process almost to a standstill.

If attempts were made to skin-graft wounds in this condition the results were very unsatisfactory, as although the small grafts employed usually become adherent there was no tendency for the epithelium to spread from them, and after a period the grafts themselves slowly disappeared. It was, therefore, decided to ascertain if the lymph flow to the granulating surface would be improved and, in consequence, the rate of healing of the wound increased, by having the mass of scar tissue and immediate neighbourhood of the wound massaged daily.

The results obtained were very satisfactory and encouraging,

the granualting surface became redder and healthier in appearance and the epithelium spread from the edges of the wound much more rapidly. The massage caused no inconvenience to the patient and could usually be carried out with considerable vigour as wounds of this type are as a rule quite painless.

(8) Vaccine therapy.

As soon as the bacteriology of a few of the cases had been studied and it had been ascertained that infection with *Streptococcus pyogenes* was extremely common, a stock vaccine was prepared from several strains of this coccus isolated from different cases, and the routine practice of giving small doses at intervals of about a week was instituted. This procedure was continued throughout the whole series of cases treated and the impression gained was that it was of

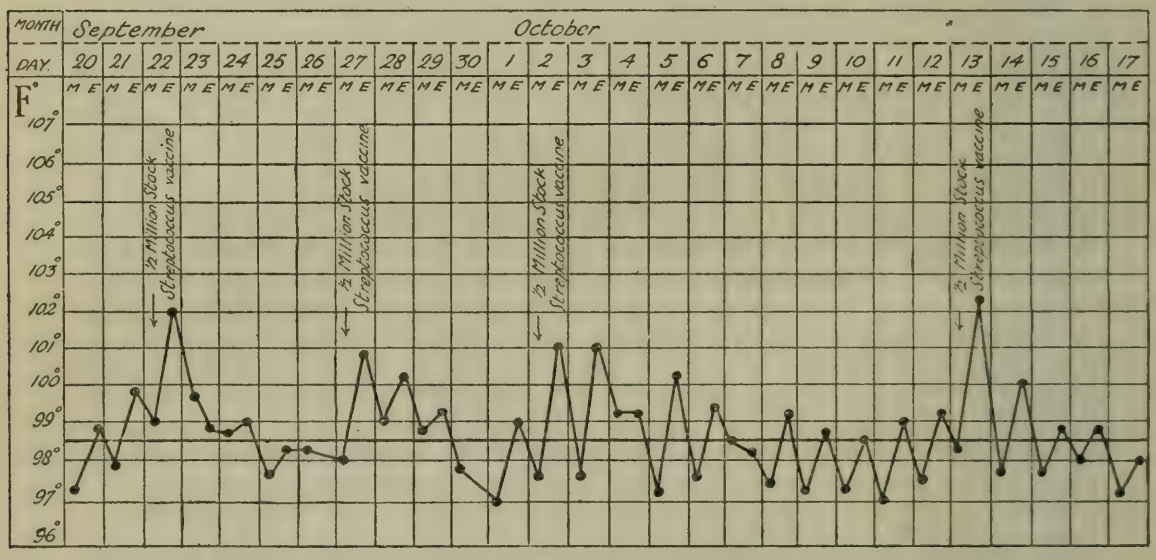


CHART I.

Portion of the temperature chart of Case No. 25; showing a slight but constant reaction after as small a dose as ½ million *Streptococcus pyogenes* vaccine.

very considerable benefit in bringing the patient's temperature down to normal and keeping it at that level, in preventing complications such as cellulitis and erysipelas and in improving the patient's general condition.

In cases where the wound was inefficiently drained vaccine therapy, as was expected, was quite ineffectual.

As regards dosage the study of certain cases appeared to show that when the wound surface was extensive, thus giving a large area of absorption, and also during the period before the surface became covered with granulations, it was necessary to use very small doses. For instance, the examination of the temperature chart of case No. 25, a patient who was suffering from an extensive and deep wound of the thigh, shows that even as small a dose as half a million cocci repeatedly caused a distinct but slight reaction a few hours after the dose was given. This case was, however, exceptional and the usual procedure was to give a dose of about 1 or 2 million cocci at intervals of from five to seven days. The dose was slowly increased

to 5 and occasionally 10 million cocci ; larger doses than the latter were not often employed as no greater benefit was gained.

Several other stock vaccines were employed on occasions, their use depending on the result of the bacteriological examinations ; for instance, *B. proteus* vaccine in doses of 5 to 10 millions, *B. pyocyaneus* vaccine in doses of 2 to 10 millions, *Streptococcus salivarius* vaccine in doses of 2 to 10 millions and *Staphylococcus* vaccine in doses of 50 to 500 millions ; all these vaccines were prepared from different strains of micro-organisms isolated from various patients.

(9) The Advantage of using an Autogenous Vaccine in certain cases.

On several occasions, in spite of vaccine treatment on routine lines, a patient's temperature or progress remained unsatisfactory

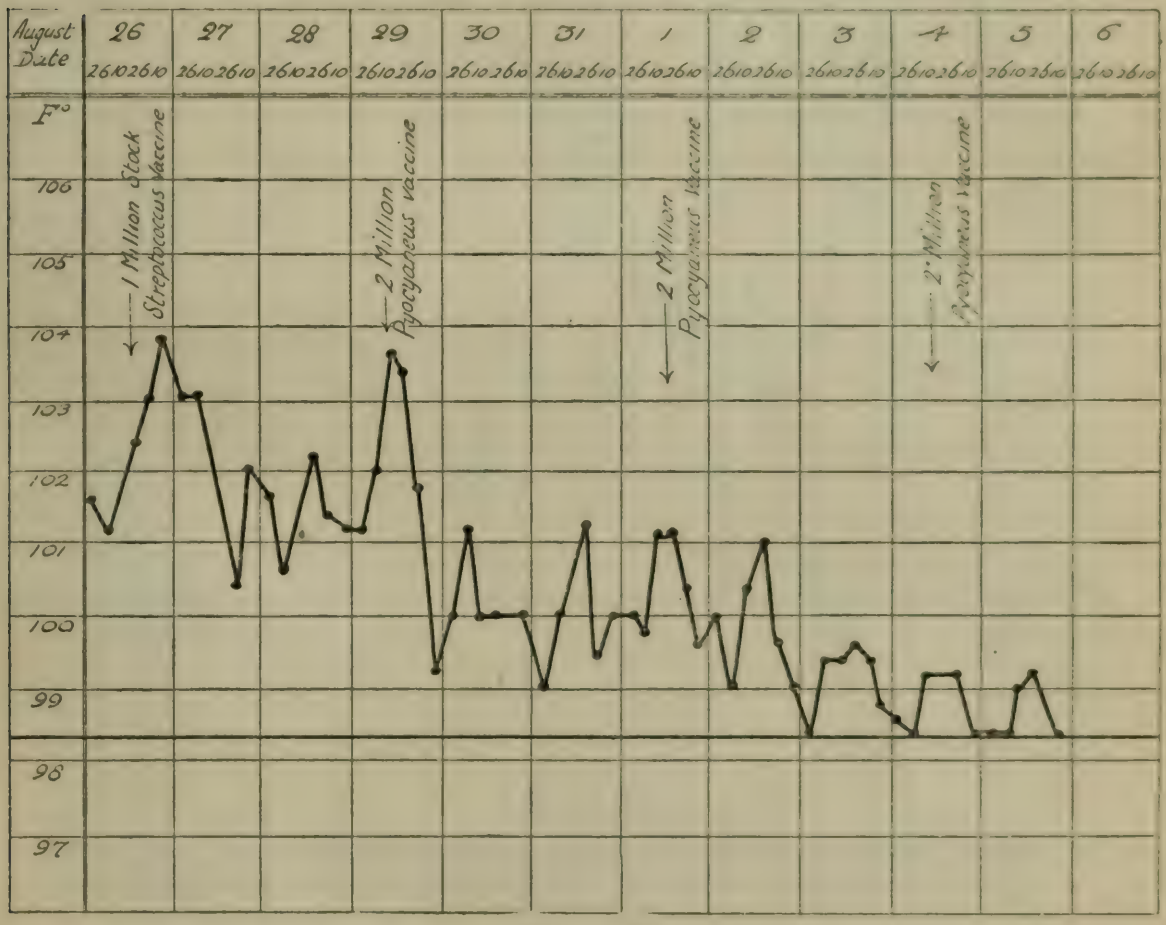


CHART II.

A portion of the temperature chart of case No. 13 showing the effect of giving small doses of *B. pyocyaneus* vaccine. The patient had had fever of the same type for the previous three weeks and had been treated without any obvious benefit with two different streptococcus vaccines.

until, as a result of careful bacteriological investigation, a different vaccine was employed, when the conditions changed for the better with almost dramatic suddenness.

For instance, case No. 13, a patient suffering from an extensive wound in the neighbourhood of the right scapula, complicated with bronchitis, continued to have a considerable amount of fever even

after the lung complications had disappeared. Inoculations of small doses of vaccines made from *Streptococcus pyogenes* which was present in the wound and from a *Streptococcus* which had grown in one out of ten tubes planted with the patient's blood, had no effect either on the temperature or on the condition of the wound, which was at this time not at all satisfactory. Cultures having shown the presence of *B. pyocyaneus* in large numbers, a small dose of vaccine prepared from the strain isolated from the patient was given. The result was most striking, as the temperature, which had

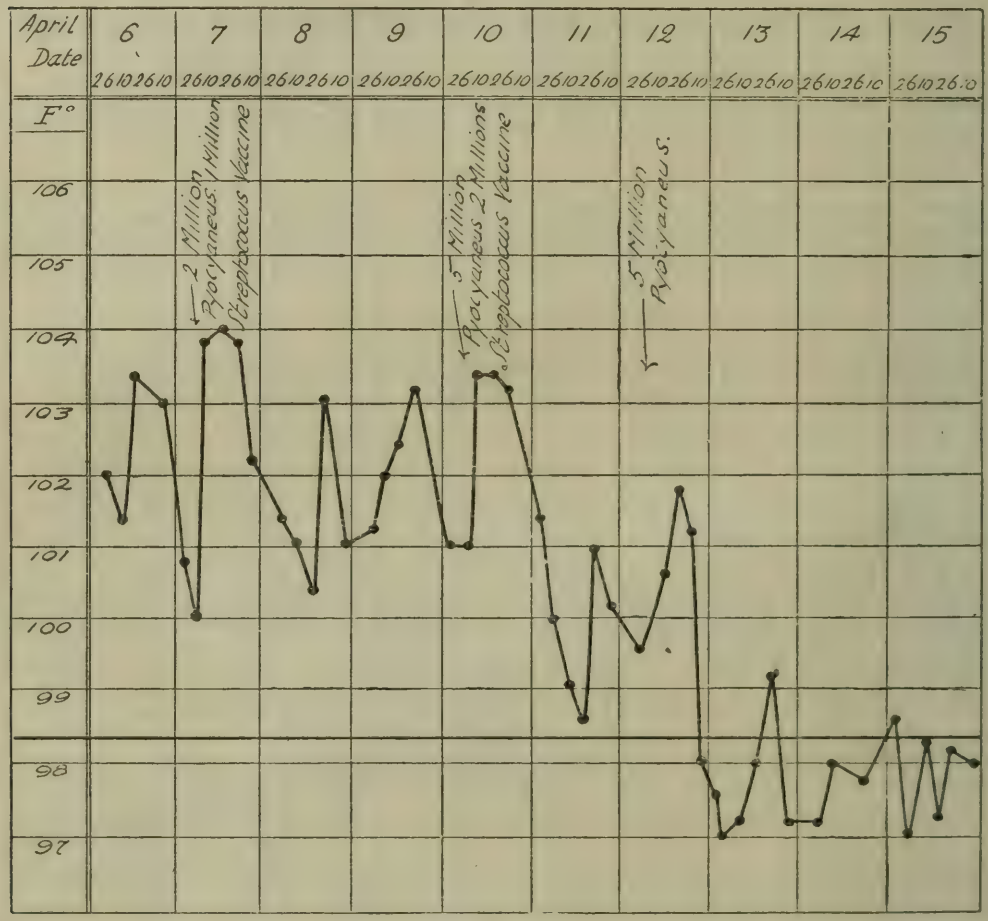


CHART III.

A portion of the temperature chart of Case No. 54 ; showing the effect of *B. pyocyaneus* vaccine. The temperature had been of the same type for the previous 8 days.

remained consistently high since the patient's admission three weeks previously, fell the same day and assumed a lower level. Three days later another small dose was given after which the temperature fell to almost normal, and throughout the remainder of the treatment fever was absent ; at the same time the appearance of the wound and the patient's general condition improved enormously. A portion of this patient's chart is given (see Chart II) showing the effect of the pyocyaneus vaccine on the temperature.

Later in the same patient's treatment a marked improvement in the condition of the wound surface was brought about by inoculating doses of a vaccine prepared from an unclassified *Streptococcus*

which cultures showed to be consistently present in very large numbers.

The next case, No. 54, demonstrates the result of giving small doses of *B. pyocyaneus* vaccine in certain cases infected with this bacillus. The patient was suffering from a fractured femur, which had been complicated with gas gangrene and was suppurating very freely. The temperature about ten days before the treatment had taken a very much higher level, and in spite of everything possible being done to improve the drainage, &c., it remained high. When

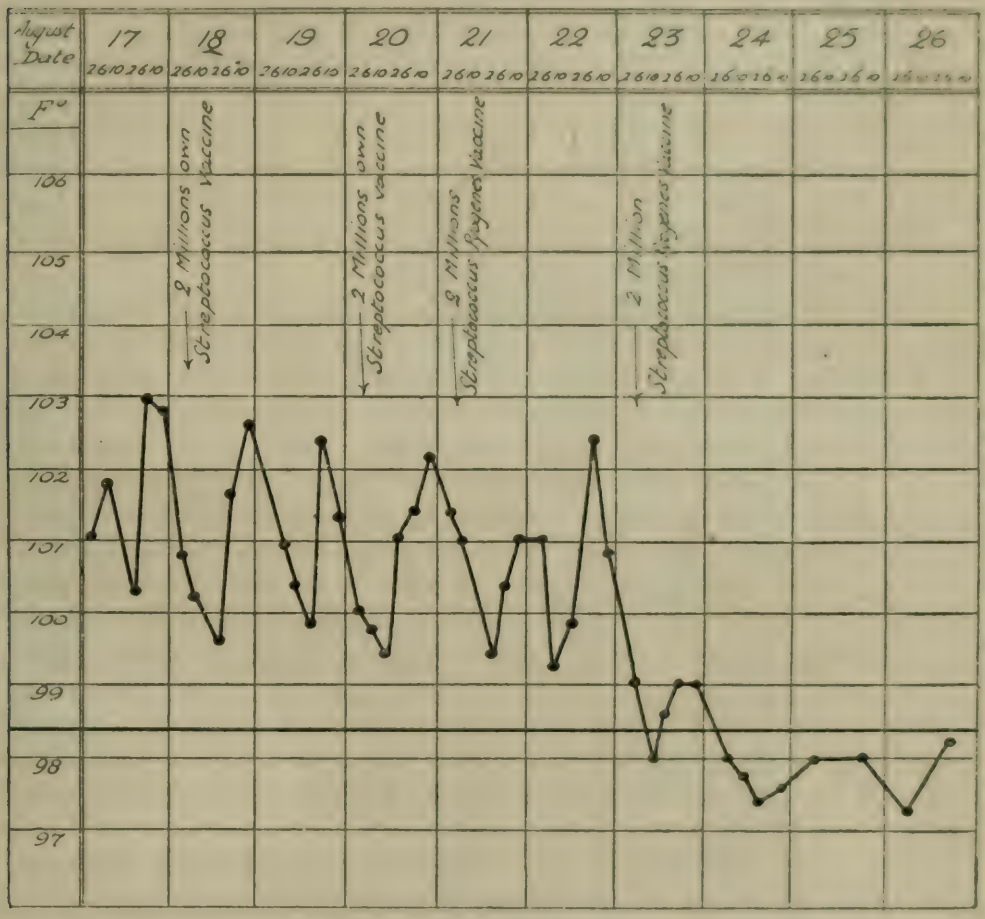


CHART IV.

A portion of the temperature chart of Case No. 8 during an attack of erysipelas, showing the rapid fall which followed the administration of *Streptococcus pyogenes* vaccine when doses of a vaccine made from another variety of Streptococcus had had no beneficial effect.

small doses were given of *B. pyocyaneus* vaccine, this bacillus being present in large numbers in the pus taken from the deeper parts of the wound, improvement was very rapid and progressive after the administration of each dose. Chart III shows the effect on the temperature, which very quickly reached the normal line. After this date fever did not return.

The last instance that will be given is case No. 8, who shortly after his admission developed erysipelas. A blood culture having given a positive result in one tube, out of the ten planted, of an unclassified *Streptococcus*, it was thought that this coccus was pro-

bably the cause of his condition. A vaccine was, therefore, prepared and small doses administered without the slightest benefit. It was, therefore, decided to give doses of *Streptococcus pyogenes* vaccine, this coccus being the common cause of erysipelas and being present in the patient's wounds. The result was most satisfactory, the temperature quickly falling to normal and remaining at that level.

(10) The operation of secondary suture.

Our small experience served, so far as it went, to confirm that obtained on a much larger scale elsewhere. In particular we learned to lay great stress upon the choice of time at which secondary suture should be performed. The moment when sloughs disappeared and the surfaces of the wound took on a raw-meat appearance generally coincided with a reduction of the bacteria to a minimum. At this time also the exposed surfaces were only too ready to unite when brought into apposition, and the newly-formed granulation tissue was still soft and supple enough to permit of closure of the wound with but very little tension on the sutures.

When the operation was performed under these optimum conditions our experience was that wounds could be successfully sutured which showed a much larger number of bacteria per field of the microscope than Carrel has laid down as permissible. We also found that the presence of streptococci did not preclude a successful result.[1] Subsequent work published by Wright, Fleming, and Colebrook throws light on these findings and the general experience of secondary suture operations. The leucocytes found in wounds are shown to have enormous power of killing all the bacteria which infect war wounds, not excepting the streptococci, but this destruction cannot be counted upon except in the following circumstances: the leucocytes must be present in large numbers and must have recently emigrated; they must be protected from the inimical influence of desiccation, and—most important of all—the bacteria must not be allowed access to pools of fluid, in which they can multiply out of reach of the leucocytes.

In an open wound there is usually no lack of fresh leucocytes and it is not difficult to give them protection from drying—on the other hand, the surface of such a wound is almost never without crevices and pockets which serve for the collection of serous fluids, and for this reason probably it is quite exceptional for such a wound to completely sterilize itself. When, however, its surfaces are brought into close apposition by sutures, not only are the leucocytes protected from desiccation and their concentration doubled at every point, but—if the operation is undertaken when the tissues are supple and very ready to unite—all the crevices and pockets, commonly referred to as dead spaces, are obliterated. The success of secondary closure operations, in our judgement, depends chiefly upon the completeness with which the dead spaces are eliminated; and this, in turn, upon the surgeon's skill in effecting a close apposition of the tissues without undue tension upon the sutures, and—a factor too little emphasized—in the application of uniform pressure by firm bandaging after the operation. If the closure is attempted

[1] Wright, Fleming, and Colebrook, *Lancet*, 1918, i. 831.

while sloughs are still adherent to the wound, they will not only constitute dead spaces in which serous fluid will collect, but also, since the sloughs are foci of bacterial growth, will ensure the pollution of the fluid and thus lead to certain failure of the operation; and the same result is to be expected if the operation is deferred until cicatrization of the tissues compels the surgeon to put undue tension upon his sutures.

One point remains to be made here: Fleming has shown by means of bio-cultures (vide Wright, Fleming, and Colebrook, *Lancet*, 1918, i. 831), made at intervals after the dressing of an open wound, that the conditions are most in favour of sterilization by the agency of leucocytes between four and eight hours after dressing. The secondary suture operation should, therefore, be undertaken during that period.

(11) The persistence of living bacteria in scar tissue and sequestra.

The question of bacteria remaining alive in scar tissue has also very important bearing on the performance of operations such as the resection of joints, the plating of fractures that have united in bad position, and the suture of nerves after suppurating wounds have healed; and not only has it been impressed on the present authors during the present investigations, but also by facts brought to their notice from time to time in the past, that streptococci and other bacteria may remain embedded in scars without producing any symptoms for months and perhaps years. These bacteria, although they may lead to no symptoms for long periods, are capable of producing very active suppuration if the scar becomes injured in any way, such as being opened up during an operation, and it is not uncommon to hear of a joint which has long before been the seat of a bacterial infection becoming intensely inflamed or even suppurating after violent movements made to break down adhesions. One of us can recall a case where a gunshot wound of the right forearm, which had been healed after much suppuration for over five months, was operated on in the hope of obtaining union in an ununited fracture of the ulna; twelve hours after the operation the wound had become very septic and an abundant culture of *Streptococcus* was obtained from the discharge.

Another point bearing on this subject brought to the notice of the same individual is, that horses used for the preparation of anti-streptococcic serum by injecting cultures into the muscles of the neck, will, months after the injections have been stopped, develop abscesses due to streptococci at the site of the former inoculations if from any cause their condition becomes poor.

During the present investigations it was found that, whenever sequestra were removed from a patient who had suffered from gas gangrene, cultures of *B. welchii* and other anaerobes and aerobes could be always obtained from the sequestrum even though the *B. welchii* and other bacteria had been absent from the discharges for many months. After the sequestra had been removed from the body and had become quite dry, the bacteria still remained alive. Thus from a sequestrum which had been removed from the body

and kept quite dry for two years, not only were spore-bearing bacilli like *B. welchii* and *B. sporogenes* easily cultivated, but non-sporing micro-organisms such as *B. pyocyaneus* and *Streptococcus* were found to be living in considerable numbers. It is probable that sequestra lying in the body will be the resting place of bacteria for very long periods, and even though the wound may heal without the sequestrum being extruded, the bacteria will remain alive and capable of producing very unwelcome effects if the surrounding tissues are in any way injured or even if the patient's general health deteriorates.

From a study of these facts it is obvious that operative interference should not be undertaken too lightly in the region of wounds which have healed after suppurating freely, and that as long a period as possible should be allowed to elapse between the healing of the wound and the carrying out of the operation. Further, as far as is possible the scar tissue should be left uninjured, the necessary incisions being made through the healthy tissue. How far the danger of suppuration following such operations could be lessened by such measures as massage of the scar, gentle at first and gradually becoming more vigorous, together with inoculation of vaccines made from *Streptococcus pyogenes*, has not been worked out, but both these procedures appear to be worthy of trial.

B. GENERAL OBSERVATIONS ON THE BACTERIA FOUND IN THE WOUNDS

(1) The faecal origin of wound infections.

The results of the bacteriological investigations on those cases which arrived in this country shortly after the infliction of the wounds showed, as had been pointed out by one of us (A. F.),[1] that the primary infection is largely made up of faecal bacteria. Wounds in this stage practically always yielded cultures of *B. welchii* and other spore-bearing anaerobes ; associated with them were *B. proteus*, *Streptococci* (generally of the *faecalis* or *enterococcus* types, but even in this stage *Streptococcus pyogenes* was sometimes found), varieties of diphtheroids, *Staphylococci*, and tetrad cocci.

As the wounds cleaned under treatment the bacteria became fewer and fewer in number and the anaerobes quickly disappeared, so that after a few days they were only to be found in sloughs and other dead tissues. When the wound had quite cleaned and had reached what has been frequently described as the ' raw meat stage ' the bacteria present in the discharge were usually extremely few in number and, as has been mentioned above, this was a very favourable period for the performance of secondary operations.

(2) The importance of Streptococcus pyogenes.

As the wound surface became covered with granulations and any cavities or irregularities were filled up with granulation tissue the number of bacteria tended rather to increase, and day by day

[1] Fleming, *Lancet*, 1915, ii. 638.

it became more and more evident that *Streptococcus pyogenes* was present in practically every septic wound and was able to resist the bactericidal action of the body fluids and cells to a much greater degree than any of the other bacteria present. It was this coccus that was responsible for nearly every complication that occurred in the cases during their stay in the wards of the Inoculation Department, the commonest of these being erysipelas, the formation and persistence of sinuses, and also such constitutional symptoms as fever.

This coccus having been once found in a wound generally remained there, though frequently in very small numbers, until the wound was completely healed. It was during the later stages of the healing process that films made from the discharge from the wound showed practically no bacteria, but on making cultures, especially if these were incubated under anaerobic conditions, one was often surprised at the number of *Streptococcus pyogenes* colonies that developed. Associated with these streptococci were usually other micro-organisms; the commonest were *Staphylococcus* and diphtheroids, but *B. proteus* and *B. pyocyaneus* were also present at times. If during the healing process the surface of the wound showed portions where the granulations had an unhealthy aspect, it was usually found that the films made from the discharge taken from unhealthy looking patches showed that the bacteria were very much more numerous in these situations than in the discharge taken from the more healthy portions of the wound.

(3) The recrudescence of bacterial infections after operations.

After such operations as amputations by the flapless method or the removal of sequestra from the neighbourhood of fractures the number of the bacteria present on the wound surfaces increased greatly, so that twenty-four hours after the operation films made from the discharge showed enormous numbers of bacilli. It was also found, especially after the removal of sequestra, that bacteria which had long been absent from the discharge again made their appearance; thus a wound in connexion with a fractured femur which contained before operation only a very few bacteria, chiefly *Staphylococcus* and *B. proteus*, two days after the operation for the removal of a sequestrum showed enormous numbers of micro-organisms, amongst which were *Streptococcus pyogenes*, diphtheroids, and *B. sporogenes*. The latter had not been found before, although numerous examinations had been made since the patient was wounded six months previously. The explanation of this phenomenon is, as has been shown above, that the bacteria contained in the cavities of sequestra remain alive but quiescent for many months, but when the site is disturbed during the operation all the bacteria present tend to proliferate and infect the surrounding tissues. Under treatment, however, they rapidly disappear and the wound returns to the former condition, in which the discharge contains only a very few bacteria, the commonest and most important being the *Streptococcus pyogenes*.

PART II

A DETAILED ACCOUNT OF THE CHARACTERISTICS OF THE VARIOUS BACTERIA ISOLATED FROM THE WOUNDS

(1) Introduction—and routine procedure employed in studying the bacterial flora of the wounds.

The routine technique employed for studying the bacteriology of the wounds was as follows :

As soon as the case was admitted samples of pus were collected and from these (i) films, and (ii) cultures were made.

(i) *The films* were stained by Gram's stain and counterstained with either neutral red or weak watery fuchsin, and the results of the examinations were so recorded as to give the conditions of the pus cells, the amount of phagocytosis, and the types of the various bacteria in order of their prevalence.

(ii) *Cultures.* Two series of cultures were made from each sample of pus, one from the unheated pus, the other from pus which had been heated to 65° C. for thirty minutes. The temperature of 65° C. was employed in preference to that of 80° C., which has been recommended in the past, as, while it was found to kill off satisfactorily all the non-sporing micro-organisms, with the exception of an occasional variety of streptococcus,[1] it did not prevent the spores from rapidly growing out when placed under favourable conditions ; whereas, when the pus had been heated to 80° C., many of the spores appeared to be so damaged that the resulting growth even on the most favourable media was very scanty. The media and type of cultures used in each series were as follows :

(*a*) Glucose agar plates.
(*b*) Milk tube.
(*c*) Trypsin-blood-broth tubes.

The glucose agar was prepared from trypsin broth described by one of us (S. R. D.[2]), to which 0·25 per cent. of glucose had been added. This trypsin broth is a much better basis for solid media than broth prepared from meat extracts and dried peptones.

The trypsin blood broth was made by adding 1 c.c. of fresh human blood and 0·25 c.c. of the Inject. Trypsin Co. (Allen & Hanbury) to 5 c.c. of the trypsin broth mentioned above. This medium has proved a very favourable one for nearly all micro-organisms, and its use has frequently enabled an abundant culture to be obtained when little or no growth developed in ordinary broth.[3, 4]

One glucose agar plate and one trypsin-blood-broth tube made

[1] It has been found that occasionally streptococci from wounds resist even a temperature of 80° C., and Simonds has shown that this also holds for some streptococci in faeces, a result which has been since confirmed.

[2] Douglas, *Lancet*, 1914, ii. 891.

[3] Douglas and Colebrook, *Lancet*, 1916, ii. 180.

[4] From observations made subsequently to those included in this paper we do not consider that this trypsin blood broth is a better medium for growing the spore bearing anaerobes than is a medium consisting of minced meat and water rendered

from the unheated pus were incubated under aerobic conditions at 37° C., the remaining glucose agar plate and trypsin blood broth tube, together with a milk tube made from the unheated pus, and the whole series of the cultures made from the heated pus were incubated under anaerobic conditions—the method used being the paladium-black fitted tins described by McIntosh and Fildes.[1] After a period of incubation the plates were examined and the various colonies picked off and planted on trypsin broth agar so as to obtain pure cultures. The milk tubes were of little use except to demonstrate the presence of *B. welchii* by the production of the typical clot in twenty-four hours.

The trypsin blood broths were planted out on to glucose agar plates, after films had been examined to give some idea of the number and variety of organisms that had developed, and the resulting plates were incubated aerobically or anaerobically according as the original fluid cultures had been incubated. After this further incubation the plates were examined and the various types of colonies were picked off and planted on to trypsin-broth-agar slopes.

Further samples of pus were collected from time to time and the various micro-organisms isolated from them. By these methods a large number of pure cultures were collected and classified. They will be discussed in detail under the following headings :

(1) The anaerobic bacteria.
(2) *Streptococci.*
(3) Diphtheroids.
(4) *B. proteus.*
(5) *B. procyaneus.*
(6) *Staphylococci.*
(7) Various coliform bacilli.

(2) The anaerobic bacteria.

(*a*) General Considerations.

Prior to 1914 the literature on the spore bearing anaerobic bacilli was voluminous but very confusing, and it has become evident that much of this confusion was due to the fact that in many cases the cultural characteristics were described from impure cultures. Thus even such a well-defined bacillus as *B. welchii* was described with characteristics quite foreign to it.

In describing the technique employed for the isolation of the various micro-organisms present in the pus, attention is drawn to two points : (i) when the pus was heated to destroy the non-sporing bacilli a temperature of 65° C. was not surpassed ; (ii) the media used for making the plates—trypsin-broth-agar—is capable of giving a much more abundant growth than that made from meat extract and commercial peptone. It is believed that attention to these details proved an important factor in obtaining pure cultures of the

slightly alkaline to litmus. This medium is very easily prepared, and it has the advantage that it reveals the colour changes produced by the action of the different microbes on the meat. The meat medium also is of the greatest use for storing the cultures.

[1] McIntosh and Fildes, *Lancet*, 1916, i. 768.

various anaerobic bacilli, since owing to a very much smaller implantation of the pus being necessary to obtain a satisfactory number of colonies on the plate, the chances of other spores being included within the limits of the colony were greatly lessened. It was found to be much easier to obtain pure cultures direct from pus heated to 65° C. than from mixed cultures obtained by planting pus into fluid media, and the latter method was only used when the plates made directly from the heated or unheated pus failed to give growths of certain bacilli which had been observed in the examination of stained films.

Before passing on to describe the various anaerobic bacilli the rôle played by them and the period during which they were present in the wound will be discussed. As has been pointed out by one of us (A. F.), the primary infection of all the wounds is largely a faecal one, and the vast majority of the micro-organisms found in the pus during the early stages of the infection consist of various anaerobic bacilli, coliform bacilli, and streptococci. They are, during the earliest stages, confined to the damaged area, multiplying with extraordinary rapidity owing, partly at any rate, to the beneficial action of symbiosis. It has been shown by the present authors[1] that, when mixed with these other bacteria, an abundant culture of *B. welchii* will often develop, although the number of bacilli implanted was 10,000 or even 100,000 times smaller than that found necessary to produce a *pure* culture in the same serum. It is during the stage of very rapid proliferation of the micro-organisms in the damaged area that gas gangrene occurs by the invasion of the tissues by certain anaerobic bacilli.

When under efficient treatment the wound is cleaned up, or when in those cases where gas gangrene had developed that process is arrested, all the anaerobic bacilli rapidly disappear, so that even in a few days they may become comparatively very few in number and have usually completely disappeared from uncomplicated flesh wounds in ten or twelve days. If, however, any bone has been injured or sloughs remain attached to the tissues, the various anaerobic bacilli can be almost invariably found, and in the cases where bone sequestra are present the common anaerobes can be isolated from the necrosed bone months or even years after the infliction of the wound. Sometimes these anaerobic bacilli cannot be recovered from the pus exuding from the wound, even when there is a sequestrum *in situ* in which they can be shown to be present.

In the further study of the anaerobic microbes the pure cultures were planted on trypsin broth agar, trypsin broth, and also on these media with the addition of glucose. In addition to the growth on these media the action of the bacilli on neutral red egg medium was noted ; the fermentation reactions were also investigated by growing the bacilli in broth prepared from casein, as suggested by Cole and Onslow[2], to which the various sugars had been added. The appearance and power to liquefy gelatine cultures was also recorded.

The neutral red egg medium was introduced by one of us (A. F.) in 1908, and is prepared exactly as is Dorset's egg medium with the

[1] Douglas, Fleming, and Colebrook, *Lancet*, 1917, i. 604.
[2] Cole and Onslow, *Lancet*, 1916, ii. 9.

addition of 1 per cent. of a 1 per cent. freshly prepared watery solution of neutral red. It should have an orange colour and a firm dry surface. The formation of acid and the action of many of the bacteria on the neutral red produce a variety of colour effects which often prove helpful in identifying micro-organisms and ascertaining if the culture is pure.

(*b*) Classification.

With one exception the anaerobic bacteria which were isolated from the various wounds can be grouped in the following three classes:

I. The non-sporing bacteria.
- (i) Cocci.
- (ii) Diphtheroids.

II. The non-putrefactive spore bearing bacteria.
- (i) *B. welchii.*
- (ii) *B. tertius.*
- (iii) *B. cochlearis.*
- (iv) *B. butyricus.*
- (v) *B. sphenoides.*
- (vi) An unnamed bacillus isolated from two cases, known as Sindrey I and Liddicoate I.
- (vii) An unnamed bacillus morphologically resembling *B. tetani* isolated on one occasion only, known as Tholby II.

The general characters of this group are that their power to digest coagulated albumens is relatively slight, while in most cases they are able to split up many of the carbohydrates commonly used in testing the fermentation reactions of bacilli. The fact that several have considerable power of splitting up uncoagulated proteids must not be lost sight of; for instance, it is widely recognized that *B. welchii* growing in serum splits up some of the proteid and produces large quantities of gas.

III. The putrefactive group of spore bearing bacteria.
- (i) *B. sporogenes.*
- (ii) An unnamed bacillus isolated on one occasion only, known as Cullingford 3 C.
- (iii) An unnamed bacillus isolated on one occasion only, known as Cullingford 3 B.
- (iv) An unnamed bacillus isolated on one occasion only, known as Cullingford X.
- (v) An unnamed bacillus isolated on one occasion only, known as McFadzean I.
- (vi) An unnamed bacillus found on one occasion, known as Hancock II.

The general characters of this group are that the bacilli have the power not only of breaking down and producing intense putrefaction in uncoagulated proteids, but also of rapidly liquefying and putrefying coagulated albumens. As a group they have little action on the carbohydrates.

There remains but one bacillus which was isolated on one occasion only and was known by the laboratory name of Boone I; this bacillus possesses characteristics which make it impossible to

place it in any of the above groups ; it appears to occupy an intermediate position.

(*c*) The Non-sporing Anaerobic Bacteria.

This group has been very imperfectly worked out, and practically nothing is known of the rôle played by it in the infection of wounds.

(i) *Cocci.*

'Forest's' anaerobic tetrad. This microbe was isolated from a glucose agar plate which had been planted directly from the pus immediately after the patient's admission to hospital. Microscopic examination showed it to be a coccus arranged in regular groups of four, or masses of such groups ; in size it was similar to *Staphylococcus* ; Gram's stain was retained. At times very many of these cocci were seen in films of the pus, each group of four being surrounded by a well-marked capsule. On agar and glucose agar slopes it grew in very minute rounded flat transparent colonies which were difficult to see without the aid of a lens. These cultures died out in four or five days. No growth ever took place under aerobic conditions, and in glucose agar shake cultures no colonies developed in the upper centimetre of the medium even after the organism had been frequently subcultured during a period of several weeks.

Tholby's anaerobic tetrad. The morphology of this organism was similar to the above. The colonies on agar and glucose agar were, however, quite unlike those of the 'Forest' organism, being round, heaped up, and very opaque in size, similar to a colony of *Streptococcus pyogenes.* This coccus retained its anaerobic properties for several generations, but later it was found to have acquired the property of growing aerobically.

Harris's anaerobic coccus. This microbe was isolated from a glucose agar plate planted directly from the pus immediately after the patient's admission to hospital. Morphologically it was a coccus, in size and shape similar to a *Staphylococcus.* Gram's stain was however *not* retained. The colonies on agar and glucose agar resembled those of *Streptococcus pyogenes.* Stab cultures gave good growth in the depth of the tube but none within 1·25 centimetres of the surface. Broth cultures showed a considerable granular deposit with slight clouding of the medium. On neutral red egg a fine growth was observed after 24 hours' incubation, but no reddening of the medium occurred. No growth took place on gelatine at 20° C. Milk was neither clotted nor rendered acid. Planted into broth containing various carbohydrates, acid was produced in that containing cane sugar, but glucose, mannite, glycerine, raffinose, salicin, inulin, dextrin, starch, and dulcite were unaffected.

(ii) *Diphtheroids.*

Sindrey's anaerobic diphtheroid. This was a long slender bacillus, Gram-positive, and non-motile. Its arrangement was of a diphtheroid character. Cultures rapidly died out and the strain was lost before it could be examined thoroughly. This was probably the same bacillus as Wright [1] and Fleming [2] described under the name of 'Wisp' Bacillus.

[1] Wright, *Brit. M. J.*, 1915, i. 629. [2] Fleming, *Lancet*, 1915, ii. 638.

(*d*) The Non-putrefactive Group of Spore-bearing Anaerobic Bacilli.

(i) *B. welchii, B. perfringens* or *B. aerogenes capsulatus.*

The ordinary cultural characteristics of this bacillus are so well known that they will be only briefly referred to. On agar the colonies are rounded, somewhat opaque with a granular texture, the edges smooth and regular. In broth an even turbidity is at first produced, but this condition is followed by a sedimentation of the bacilli which, after a few days, leaves the supernatant broth quite clear. Milk is rapidly clotted, the clot being of a peculiar spongy texture due to its being broken up by gas formation. No digestion



Fig. I. Forty-eight hours culture of *B. welchii* in serum.

of the clot ever occurs. On neutral red egg bright yellow colonies are formed in 24 hours, the surrounding medium being crimson; this is due to the bacillus destroying the dye in the immediate neighbourhood of the colony while the surrounding red colour is due to the formation of acid. Even after months no digestion of the egg can be demonstrated.

Morphologically the bacillus is a short stout rod with slightly rounded ends, retaining Gram's stain. It is non-motile. Its length varies greatly according to the media on which it is grown: thus films made from cultures grown on egg medium show a bacillus almost as broad as it is long, those from cultures on agar are considerably longer, while the blood agar cultures show still longer organisms. No growth takes place on gelatine at 20° C. Fig. I shows the appearance of a culture grown in serum for 48 hours.

Spore formation. It has been commonly stated that *B. welchii* is incapable of forming spores when cultivated in a medium con-

taining a carbohydrate which is fermented with the production of acid during the growth of the bacillus.

The present authors have been unable to demonstrate microscopically spores in young cultures grown either on broth or agar, but such cultures were not sterilized by exposure to a temperature of 60° C.—a temperature quite sufficient to kill the vegetative forms, so it must be presumed that spores are formed. In broth containing 0·5 per cent. glucose spore formation could never be detected, and such cultures were invariably rendered sterile by heating them to 60° C. for one hour.

When investigating the fermentation reaction of this bacillus, films were made from the various cultures and examined microscopically ; it was then found that the bacilli grown in broth containing 1 per cent. starch showed very abundant spore formation although the



Fig. II. Twenty-four hours culture of *B. welchii* in starch broth.

media gave a very strong acid reaction. This phenomenon was a constant one in the six strains that were examined, but the number of bacilli forming spores varied considerably. In three of the strains sporulation was extremely abundant, so that in 24 hours at least half the bacilli could be demonstrated by staining to have formed spores. See Fig. II.

The remaining three strains showed a somewhat different picture, most of the bacilli having become swollen and staining in an irregular manner so that portions of the bacillus remained uncoloured. At times these unstained portions were oval in shape and simulated a spore, but they did not retain the spore stain. Fig. III shows the appearance of these organisms. In every culture, however, some typical spores could be demonstrated by staining, and in the case of all the six strains starch broth cultures 5 months old when heated to 80° C. gave abundant growth when planted into milk, showing conclusively that many spores had been formed. In broth con-



Fig. III. 48 hours' culture of *B. welchii* in potato broth, showing irregular staining.

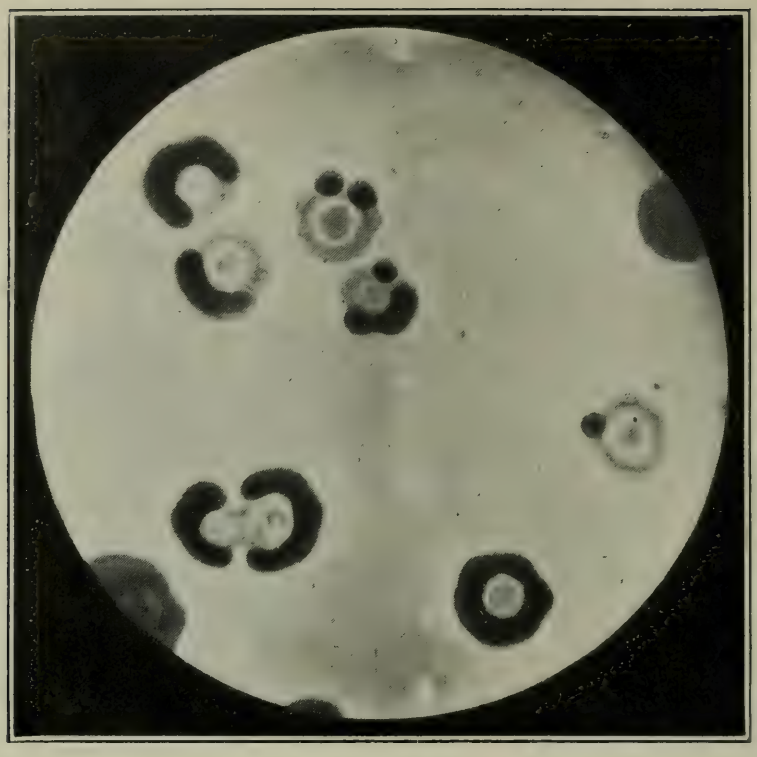


FIG. IV. Atypical colonies of *B. welchii* on agar. These were obtained from one strain only.



FIG. V. Atypical forms of *B. welchii*—the same strain as that shown in Fig. IV.

taining 1 per cent. dextrin the bacilli assumed the same swollen appearance and irregular staining properties, but spore formation could not be demonstrated.

One of us (S.R.D.) has shown that spore formation is very rapid and profuse when *B. welchii* is grown in serum symbiotically with *Staphylococcus*, and Simonds observed spore formation even in media containing carbohydrates capable of fermentation, when the bacilli were grown in symbiosis with other faecal micro-organisms. Some spores can always be demonstrated in serum cultures several days old.

Viability. Cultures in glucose broth, milk, or on glucose agar die out in about a week. Even in ordinary broth most of the cultures were found to be incapable of subculture after two or three weeks, but marked exceptions of this rule were noticed; for instance a broth culture made in Boulogne in 1914 gave an abundant growth when planted on neutral red egg medium exactly two years later. Cultures on serum, broth containing starch, on solidified egg and any other medium containing a large proportion of albumen remain capable of being subcultured for very long periods owing to spores being formed.

The spores appear to be very resistant. Thus a culture was obtained from a vaccine made three years previously by heating a 48-hour culture to 60° C. for one hour and then adding 0·5 per cent. carbolic acid.

One strain of *B. welchii* which was isolated from one of the cases deserves special mention on account of peculiarities both as regards the morphology of the bacillus and the appearance of the colonies. These, instead of being evenly rounded and granular in appearance, showed a marked thickening at the edge, which usually commenced at a certain point of the periphery, and spread all round, leaving the centre thin and much more transparent. Fig. IV shows the appearance of such colonies.

Morphologically the bacilli after 24 hours' growth appeared larger than normal and with the end more rounded. The length and thickness of individuals varied, but long forms were more common than usual, curved forms were frequent, and there was a tendency to form chains. There were also present many large crescent-shaped deeply staining forms, and bacilli could be seen in all stages from normally shaped organisms to these remarkable forms. The crescent-shaped organisms were probably involuted bacilli. They are shown in Fig. V together with stages of transition between this condition and the normal forms.

When the culture was examined by dark ground illumination many of the bacilli were seen to have four or five bright refractile spots imbedded in them, which varied considerably in size, sometimes even distending the bacillary body. If such a specimen was stained by the common method used to demonstrate spores the refractile spots retained a pale pink colour, but an emulsion of these bacilli exposed for an hour to a temperature of 60° C. became sterile, thus proving that the spots were not true spores. Apart from the above peculiarities this organism gave all the fermentation reactions of *B. welchii* and also the typical clot in milk cultures.

Incidence. *B. welchii* was isolated from fourteen of the 61 cases investigated. The number of days after the infliction of the wound that the bacillus was isolated, a positive or negative history of gas gangrene, and the presence or absence of bone injury are in each case given below :

TABLE I.

Case.	*No. of days after infliction of wound that bacillus was isolated.*	*History of gas gangrene.*	*Presence or absence of injury to bone.*
No. 10	6	Negative	—
„ 11	6 and 55	Negative	—
„ 13	5	Positive	+
„ 29	22 from sequestrum	Positive	+
„ 34	8	Positive	+
„ 38	8	Negative	—
„ 39	8	Positive	—
„ 43	150 from sequestrum only	Negative	+
„ 45	13	Positive	—
„ 48	28	Positive	+
„ 53	13	Positive	—
„ 54	26	Positive	+
„ 56	31	Positive	+
„ 60	7	Positive	—

It is to be noticed that in the case No. 29 who had a very extensive wound of the buttock involving the whole right sacro-iliac joint and who had had a gas infection, the pus from the wound obtained 15 days after the infliction showed neither by film nor culture any *B. welchii.* On the 22nd day a large sequestrum was removed and from this *B. welchii* was readily isolated. This sequestrum was allowed to dry at room temperature, and three years later cultures both of *Streptococcus pyogenes* and *B. welchii* were readily obtained.

Fermentation Reactions of B. welchii.

The following table gives the reactions of six strains :

TABLE II.

CARBOHYDRATES.

Name of Strain.	*Glucose.*	*Lactose.*	*Mannite.*	*Saccharose.*	*Glycerine.*	*Raffinose.*	*Salicin.*	*Dextrin.*	*Starch.*	*Inulin.*	*Dulcite.*
Laboratory stock.	+	+	o	+	+	+	? o	+	+	+	o
MacFadzean	+	+	o	+	+	o	o	+	+	o	o
Grout	+	+	o	+	+	o	o	+	+	gas only.	o
Cation ..	+	+	o	+	+	o	o	+	+	gas only.	o
Hancock ..	+	+	o	+	+	—	o	+	+	+	o
Lockhart ..	+	+	o	+	+	—	o	+	+	gas only.	o

The sign + indicates the production of acid and gas ; o that the carbohydrate was unaffected ; — that the test was not carried out.

Two of the strains fermented inulin with the production of acid and gas, three other strains produced a quantity of gas in the presence of inulin without the production of any acid being observed. Mannite, salicin, dulcite, and raffinose were not fermented by any of the strains.

(ii) *B. tertius.*

This name was applied by Henry[1] to the bacillus as it was the third most common in his first series of cases. (It has since, however, been shown that it is not in reality the third commonest in these wounds, so the title is somewhat inapt, although it is more satisfactory than the various titles under which it has been described in the past, viz. B. Hibler IX, B. Rodella III, and B.Y. (Fleming).)

Hibler[2] believed that this organism was a cause of emphysematous gangrene, but although it is present in the lesions of a number of



Fig. VI. Ten days culture of *B. tertius.*

cases suffering from gas gangrene there is very little evidence that it is the causative agent.

In the past *B. tertius* has been not infrequently mistaken for *B. tetani* when attempts were made to diagnose the presence of the latter by film preparations. The principal points in which it differs from *B. tetani* is that the spore is very large and *oval* and has a much thinner ' capsule ' and that the bacillary bodies are more slender and frequently curved.

Morphology. The bacillus is long and thin, sometimes curved and sometimes straight. It is motile and retains Gram's stain. It spores rapidly on all media after 48 hours' incubation, sporing forms being very abundant. The spores are situated terminally and are very large, being much broader than the body of the bacillus. Fig. VI shows the appearance of a ten-day old culture on egg medium.

[1] Henry, *J. Path. & Bacteriol.*, 1916–17, **21**, 344. [2] Hibler, Jena, 1908.

Cultural characteristics. On agar after 48 hours' incubation the colonies are round, semi-transparent discs up to 2 mm. in diameter with slightly heaped-up centres and a marginal area slightly but distinctly crenated. Broth cultures frequently fail to develop, but when successful the medium is evenly clouded. On red egg small yellow colonies are developed on a brick-red field, the colour contrasts being much less striking than in the case of *B. welchii*. There is no trace of digestion of the albumen even after 5 or 6 weeks' incubation. Stab cultures in gelatin after 10 days' incubation at 20°–22° C. give a beautiful arborescent growth simulating the traditional growth of *B. tetani* in this medium. No liquefaction and no gas formation were observed. Milk cultures are rendered acid in 24 hours, and this is later followed by a firm, uniform clot. Gas formation is not obvious and the clot remains undigested.

Fermentation reactions.

Table III gives the results obtained with three strains:

TABLE III.

Name of Strain.	*Glucose.*	*Lactose.*	*Mannite.*	*Saccharose.*	*Inulin.*	*Glycerine.*	*Dextrin.*	*Starch.*	*Salicin.*	*Dulcite.*	*Raffinose.*
Forrest I	+	+	o	+	o	acid in 24 hrs. alk. in 48 hrs.	—	o	+	o	o
Cullingford VIII	+	+	+	+	o	+	+	+	+	o	o
Bloor	+	+	o	+	o	o	+	+	+	o	acid in 24 hrs. alk. in 48 hrs.

It is to be noted that the first strain, Forrest I, has rather less power of splitting up the carbohydrates than the other two. Further, that two strains, one in the case of glycerine and the other of raffinose, produced an acid reaction at the end of 24 hours, which was replaced by an akaline one after 48 hours' incubation.

Incidence. *B. tertius* was obtained from eleven cases out of the 61 investigated; the period that had elapsed since the wounds had been received, the positive or negative history of gas gangrene, and the presence or absence of bone injury are given in the following table:

TABLE IV.

Case.	*No. of days after infliction of wound that bacillus was isolated.*	*History of gas gangrene.*	*Presence or absence of injury to bone.*
No. 1	17	Positive	–
„ 2	9 and 14	Positive	–
„ 5	19	Negative	–
„ 8	11	Positive	–
„ 12	5	Positive	–
„ 20	77, 146, 151, and 302; the last 3 were from sequestrum.	Positive	+
„ 29	15 and 22 from sequestrum	Positive	+
„ 32	61	Positive	–
„ 34	8	Positive	–
„ 38	8	Negative	–
„ 39	8	Positive	–

In films of pus it appeared generally as long thin straight or curved bacilli, only an occasional spore-bearing form being seen; in two cases, however, such spore-bearing forms were very numerous, and in films made from a small slough from one case the appearance of the film resembled a preparation made from a pure culture.

(iii) *B. cochlearis.*

This name has been applied to the bacillus because of the likeness in shape of the fully developed sporing bacillus to a spoon. It was isolated from one patient (case no. 32).

Morphological characters. In appearance it is very similar to *B. tertius*, being a long, slender, Gram-positive, motile bacillus which forms large oval terminal spores. The spores are formed with less freedom than in the case of *B. tertius*, so that in a 48-hour agar culture very few are to be seen, although after ten days sporulation was abundant. The bacillus does not, like so many of the anaerobic spore-bearing bacilli, degenerate soon after the spore is formed; thus for a considerable period large numbers of fully developed sporing forms are to be seen which resemble straight-handled spoons. It is actively motile and this is in quite sharp contrast to the sluggish motility of *B. tertius*.

Cultural characters. The growth is relatively sparse on all media. On agar the colony is small, flat, and very transparent with slightly crenated edges. It shows no tendency to spread over the surface of the medium. In broth growth takes place slowly and produces an even clouding of the medium. On coagulated egg medium no digestion is visible and there is no putrefactive smell. In milk there is no clotting or acid formation.

Fermentations. This organism fermented none of the test sugars. In this it differs markedly from *B. tertius*, which is a powerful carbohydrate fermenter.

[In three cases a bacillus was isolated which, when grown anaerobically, gave a colony on agar and also film preparations exactly similar to *B. tertius*. (Fig. VII b). This bacillus, however, grew as well aerobically as anaerobically. In all three cases it was found associated with *B. tertius*. It fermented glucose, lactose, saccharose,

dextrin, starch, salicin, mannite, and glycerin with the formation of acid and a small quantity of gas.]



FIG. VII a. *B. cochlearis.*



FIG. VII b. Bacilli resembling *B. tertius* but growing aerobically.

(iv) *B. butyricus.*

This bacillus was isolated on one occasion only from a patient who had received a severe wound of the thigh complicated with

a compound fracture of the femur sixteen days previously. Gas gangrene developed shortly after the infliction of the wound but had been arrested by extensive incisions.

Its general characters are very irregular, but it always has the power of fermenting many carbohydrates with abundant gas formation.

Morphology. It is a medium sized Gram-positive bacillus, somewhat fusiform in shape, with blunted ends. It forms spores rapidly on all media; the spore is large and oval, situated in the centre of the body or rather nearer one end. In most media the bacilli are non-motile, but a young culture in minced meat medium showed actively moving bacilli. In preparations made from cultures on egg medium the bacillus is constantly seen to take the form of threads, so long as to stretch across the whole field of the microscope. These threads show no signs of division into segments, but large irregular fusiform swellings can be noticed at intervals; these do not retain the usual spore stains.

Cultural characters. On agar round, semi-opaque colonies were produced in size up to 2 mm. in diameter. These colonies showed but little tendency to spread, but if planted thickly a confluent growth was formed. In broth and glucose broth a copious, even cloud was developed in 24 hours; later multiplication ceased, and the bacilli settled to the bottom of the tube, leaving the supernatant fluid quite clear. On neutral red egg the growth resembled that of *B. welchii.*

In milk both acid and clot were produced, and the appearance of the culture was very similar to one of *B. welchii*; microscopical examination, however, showed that abundant spore formation had taken place, which would at once differentiate the bacillus from *B. welchii*, which never forms spores in milk.

Gelatin stab cultures incubated at 20° to 22° C. show at first a white line of growth along the track of the needle with some irregular prolongations into the surrounding medium. Later the gelatin is broken up by gas formation. No liquefaction was ever seen.

The following *fermentation reactions* were recorded: glucose, lactose, saccharose, glycerin, starch, raffinose, and salicin were all fermented with the production of acid and gas. Mannite, dulcite, and inulin were unaffected.

This bacillus appears to be quite unimportant, the only point which should be borne in mind is that the naked eye appearance of the milk culture is very similar to that of *B. welchii.*

(v) *B. sphenoides.*

This name has been given to a bacillus, isolated from wounds, on account of the wedge-shaped appearance of the fully developed sporing bacillus.

Morphology. This is a small motile Gram-negative bacillus, fusiform in shape and often arranged in pairs placed end to end. The spore when first formed is subterminal but as it enlarges it takes a terminal position; in shape it is perfectly round and of large size,

being considerably broader than the body of the bacillus. When the spore reaches its maximum development the body of the bacillus is at first seen to be wedge-shaped, tapering from the spore to a point; later the body contracts near the spore so that the appearance becomes more drum-stick-like, but the end opposite the spore always remains pointed.

Cultural characteristics. On agar the colony is small and rounded or very slightly irregular, about 1 mm. in diameter. In broth growth is profuse, an even cloud being produced. On neutral red egg medium, at first yellow colonies surrounded by a brick red area are seen, at the end of a week the whole surface becomes brick-red. In milk, acid is produced in 24 hours, a day or two later clotting takes place. The clot is firm and contracts very little. One strain produced acid in 48 hours, but no clotting took place even after six weeks' incubation. In gelatine stab cultures incubated at 20° to 22° C. rather small woolly colonies are produced along the needle track. After further incubation, the medium around these colonies is blackened, producing a very characteristic appearance. Fig. X shows this condition. There is no liquefaction of the gelatine.

The *fermentation reactions* of three strains are shown in the following table:

Table V.

Name of Strain.	*Glucose.*	*Lactose.*	*Mannite.*	*Saccharose.*	*Inulin.*	*Glycerine.*	*Dextrin.*	*Starch.*	*Salicin.*	*Dulcite.*	*Milk.*
Ingram I	+	+	+	+	o	o	+	+	+	o	Acid. Clot.
Tholby IV	+	+	+	+	o	o	+	+	+	o	Acid. Clot.
Kilby I	+	+	o	o	o	o	−	o	+	o	Acid. No clot.

It is to be noted that the third strain, Kilby I, had a markedly inferior power of splitting up the various sugars, &c., employed in these tests.

Incidence. *B. sphenoides* was recorded as present in five cases, but was only isolated in pure culture from three. All these patients had had gas gangrene. The following table gives the number of days that elapsed between the receipt of the wound and the finding of the bacillus:

Table VI.

Case.	*Period that elapsed from infliction of wound to finding of bacillus.*
No. 9	25 days
„ 21	16 days
„ 4	24 days
„ 20	5 months (from sequestrum)
„ 39	8 days

Pathogenicity. No experiments have been made to determine the pathogenic properties of these bacilli.



FIG. VIII. Forty-eight hours culture of *B. sphenoides* in starch broth.



FIG. IX. Forty-eight hours culture of *B. sphenoides* in potato broth.

(vi) *An unnamed bacillus isolated from two cases, known as Sindrey I, or Liddicoate I.*

Morphology. The average size of this bacillus is similar to that of *B. sporogenes*; there are, however, some long forms always present in preparations made from cultures. The ends of the bacillus are square; Gram's stain is positive; no motility was ever observed. Spore formation is rapid, cultures 24 hours old showing large numbers. The individual spores are very large and oval in shape, and are either central or subterminal in position, occasionally a large spore appears to be terminally situated.

Cultural characters. On agar the colonies are very flat and translucent and show a marked tendency to spread over the medium so as to form an almost imperceptible film which shows a wavy, ragged edge. If the surface of the medium is very dry the colonies remain separate and are then seen to have a very small opalescent centre from which several flat branching processes radiate; these colonies resemble in some respects one of the forms taken by colonies of *B. sporogenes*, but they are much more transparent and the radiating processes are relatively much longer. In broth cultures growth is at first very rapid, leading to the medium becoming evenly clouded. Very soon, however, the bacilli and spores tend to settle to the bottom, leaving the supernatant broth quite clear. Milk cultures showed no change in 48 hours. On gelatin incubated at 20° C. no growth occurred. On red egg growth takes the form of a flat film which bleaches the medium underneath it, the surrounding medium takes on a red colour. After a week's incubation the medium begins to fissure, and there is an increase of the water of condensation, indicating a slight digestion of the coagulated albumen. Digestion does not, however, advance beyond this stage, even after the cultures have been incubated for a month. In fluid cultures containing pieces of meat there was no apparent digestion, the meat was not blackened, and no smell of putrefaction could be detected.

Fermentation reactions. Glucose and lactose were both fermented, acid and gas being formed; in the case of the bacillus known as Sindrey I, acid was also produced in broth containing salicin. Neither bacillus had any action on the other carbohydrates which were tested.

(vii) *An unnamed bacillus isolated from one case only, morphologically resembling B. tetani, known as Tholby I.*

Morphology. The bacillus is a long one with square ends, it retains Gram's stain and in young cultures shows motility; after forty-eight hours' growth many of the bacilli show a large round terminal spore and at this stage their appearance was identical with that of *B. tetanus*.

Cultural characteristics. It was only with the greatest difficulty that a pure culture of this bacillus was obtained, for, owing to its habit of spreading over the surface, and to other factors, the cultures were always found to be mixed with *B. sporogenes*. On agar, isolated



FIG. X. Blackening of gelatine around stab culture of *B. sphaericus*.

colonies were only obtained on a medium the surface of which had been allowed to dry very thoroughly; these appeared as round, very transparent, structureless plaques. If the surface was even slightly moist the growth spread over the whole surface and appeared as a very transparent scum which was almost invisible, and unless the plate was very carefully examined, escaped notice. A gelatine stab culture incubated at 20° C. gave no growth. On red egg the growth appeared as a film. There was no evident digestion of the coagulated proteid, but the medium became fissured and the water of condensation became slightly increased. This condition remained unchanged even after the culture had been incubated for one month. Milk cultures showed production of neither acid nor clot.

Fermentation reactions. These were tested, but the resulting growths were very unsatisfactory, so that the results were not recorded.

When grown in serum this bacillus produced large quantities of gas.

Although morphologically identical with *B. tetani* many of its cultural characteristics were utterly unlike that bacillus; further, neither the living cultures nor the broth in which it had been grown showed any pathogenic effect on guinea-pigs.

(*e*) The Putrefactive Group of Spore-bearing Anaerobic Bacilli.

The outstanding character of bacilli belonging to this group is their power to rapidly break down proteid material, evolving in the process gasses with an intensely putrid smell; on the other hand, their power to ferment the various sugars, &c., is very limited.

They were by far the commonest anaerobic bacilli found in the wounds.

The classification of bacilli of this group has been rendered extremely difficult by reason of the very unsatisfactory nomenclature and confusing descriptions of the different bacteria in the literature, and when this research was conducted in 1916–17 there was little help to be obtained from the older work. The greatest confusion arose in connexion with the identification of *B. sporogenes*, *B. oedematis maligni*, and *Vibrion septique*. Many authors seemed to regard these as different names for the common putrefactive organism of wounds, but it was clear that *Vibrion septique* and *B. sporogenes* were different organisms, and although it was probable that *B. oedematis maligni* of Koch was identical with one of these it was not very evident from the literature whether it was the common putrefactive organism of the wounds or whether it was the more pathogenic *Vibrion septique*. One source of trouble has been that *Vibrion septique* has been described as possessing putrefactive properties, whereas it is now abundantly clear that it is not a putrefactive organism. Doubtless these putrefactive qualities have been ascribed to it owing to the use of mixed cultures of *Vibrion septique* and *B. sporogenes*.

(i) *B. sporogenes.*

Morphology. The bacillus is usually straight, in length similar to an average sized *B. welchii,* but very distinctly thinner than that organism; occasionally curved forms are seen (Fig. XII). It is motile and retains Gram's stain when the preparations are made from young cultures, but even after 48 hours many of the bacilli have already lost their Gram-positive properties.

Spores are formed rapidly in all cultures: they are large, bulging the bacillary body and oval in outline. As regards the appearance of the fully developed spore-bearing bacillus the different strains seemed to divide themselves up into two more or less distinct types. In the first and common type the spore is definitely sub-



Fig. XI. *B. sporogenes.*

terminal and occasional central spores are to be seen (see Fig .XI), while in the second and less common type the spore, which is proportionally rather smaller, appears at first sight to be quite terminal (Fig. XII). On closer examination, however, there is seen to be a very slight thickening of the spore envelope on the end distal to the bacillus, so that this is in reality a subterminal spore. The bacilli of this second type also are usually longer and many curved forms are to be seen. When stained for spores (with carbol fuchsin and methylene blue) the blue stain in the bacillary body was always seen to be more intense close to the spore and at the other end of the bacillus, leaving the central portion only faintly stained. In cultures 18 hours old spores are comparatively few in number, but after 48 hours they are very numerous; soon after the spores are formed the bacillary bodies degenerate, leaving only free spores.

The motility is best seen in young cultures, but even sporing forms are frequently still motile, progression taking place with the spore either in front or behind.



FIG. XIII *a*. Medusa-head type of colonies of *B. sporogenes*.



FIG. XIII *b*. *B. sporogenes*, flower-like colonies.

Cultural characteristics. The traditional form of a colony grown on agar, or glucose agar, is the so-called 'medusa head' type, which consists of a thickened opaque whitish central spot from which radiate a number of fine wavy tangled hairy processes (Fig. XIIIa). This type of surface colony can only be produced if the surface of the agar is thoroughly dried. If the agar remains moist, as it commonly does in the case of freshly poured plates, the colonies show quite different characters and have the appearance shown in Fig. XIIIb. The centre portion still remains rather heaped up and opaque, and from it thin flat opalescent processes spread over the agar in various directions, giving the colony an appearance which has been likened to a flower. Eventually, if the plate remains sufficiently moist, the whole surface will become covered with a fine opalescent film of growth due to these processes coalescing with one another, the thickened central portions of the original colony being still apparent as mere opaque



Fig. XII. *B. sporogenes.*

spots. That the very marked differences in the appearance of the colonies are entirely due to the moistness of the agar surface is proved by the fact that with a little manipulation both types of colony can be produced from the same culture. Another peculiarity of certain strains was a tendency to send prolongations into the depth of the agar, and from the pus of one case two types of colonies were constantly produced, one of which sent prolongations into the depth of the agar, while the other did not. Subcultures from these types of colony preserved their characters, but no other differences could be detected in the bacilli.

In broth *B. sporogenes* gives an evenly turbid growth. In gelatine stab cultures incubated at 20° C. growth rapidly takes place along the track of the needle; very soon, however, liquefaction commences and quantities of gas are formed; eventually the whole mass becomes liquid. In milk the most noticeable feature is the digestion of the medium, but if it is carefully examined after 4 or 5 days' incubation, when the upper portion of the milk has become clear, it is seen that the milk has been clotted, so that a mould of the tube has been

made, and this clot is, in time, completely digested. Occasionally this clotting was not observed, but the vast majority of the strains examined constantly produced it. The milk is at first alkaline, but after 3 or 4 weeks it becomes distinctly acid.

On neutral red egg medium the colony first appears as a bright yellow spot on an orange-red background; in 48 hours the medium shows distinct cupping in the immediate neighbourhood of the colony and around this depression is a blackened ring. Later the digestion of the albumen becomes more marked, so that the colonies are represented by pits containing a semi-fluid, foul-smelling material. The medium immediately around these pits is blackened. Eventually in 8 or 10 days the whole medium becomes liquefied, forming a multi-coloured, stinking mass. At the end of a month all trace of blackening has disappeared and the medium has become distinctly acid.

Fermentation reactions. Glucose was constantly fermented by all the strains with the production of acid and gas. Some strains produced acid in glycerin broth in 24 hours, but after 48 hours the medium was again found to be alkaline; all the strains produced some gas from glycerine. Many strains produced a trace of gas in broths containing dextrin and starch, and a few from salicin broth, but no acid production was ever observed. Lactose, mannite, saccharose, inulin, dulcite, and raffinose were quite unaffected.

Incidence. Bacilli of this type were isolated from 23 cases out of the 61 investigated, and in 7 it was the only anaerobic bacillus found. Of 20 strains investigated fourteen were of the type producing subterminal spores, the remaining 6 producing end spores. The number of days after the infliction of the wound that the bacillus was isolated, a positive or negative history of gas gangrene, and the presence or absence of bone injury in each case are given in the following table:

TABLE VII.

Case.	*No. of days after infliction of wound that bacillus was isolated.*	*History of gas gangrene.*	*Presence or absence of injury to bones.*
No. 1	17, 18, 32, and 46	Positive	+
,, 2	9 and 14	Positive	+
,, 4	24	Positive	—
,, 5	19	Negative	+
,, 6	10 and 37	Positive	+
,, 8	11	Positive	—
,, 12	5	Positive	—
,, 13	5 and 22	Positive	+
,, 14	16	Positive	+
,, 20	49, 77, 146, 151, and 302; the last 4 from sequestrum.	Positive	+
,, 21	17	Positive	—
,, 22	28 and 198 from sequestrum	Negative	+
,, 23	9	Positive	—
,, 24	20 and 25	Negative	+
,, 25	7	Positive	—
,, 29	15 and 22 from sequestrum	Positive	+
,, 30	16	Positive	—
,, 32	61	Positive	+
,, 33	11	Positive	+
,, 35	10	Positive	+
,, 38	8	Negative	—
,, 39	8	Positive	—
,, 50	19	Positive	+

Serum tests. It was found that by inoculating rabbits with vaccines and living cultures of *B. sporogenes* and some of the other members of this group good agglutinating sera could easily be obtained. The doses used for this purpose were, for the first dose, 500 million bacilli which had been heated to 60° C. for 1 hour, given subcutaneously; after an interval of 10 days 1,000 million living bacilli were given intravenously. By such means the serum gained the power of agglutinating emulsions of this bacillus up to a dilution of 1 in 2,000 to 1 in 3,000. In order to find out if *B. sporogenes* could be split up into a number of strains by agglutination tests, a number of preliminary experiments were carried out.

Two rabbits were inoculated, each with a different strain of *B. sporogenes*, the one being the smaller type with a subterminal spore—the most common form—the other being the longer type with an apparently terminal spore. The rabbits were inoculated with doses as described above, and after a suitable interval they were bled and the agglutination tests carried out. It was then found that the serum from each rabbit agglutinated the homologous bacilli. Further it was found that the serum of the rabbit immunized with the longer and pseudo-terminal sporing type of bacilli was able to agglutinate to a comparative titre some of the other strains having these characteristics, but had no effect on strains of the shorter subterminal spore type. The serum prepared by inoculations of the shorter subterminal spore type agglutinated in high dilutions most of the strains having the same characteristics, and at the same time were able to agglutinate—but only in dilutions of 1 in 100 and 1 in 200—some of the strains belonging to the longer end-sporing type. Several strains, however, were not agglutinated by either of these sera, and many more experiments would have to be carried out before it would be possible to decide into how many types this group of bacilli should be divided. The preliminary experiments, however, show definitely that there are at least three varieties. Unfortunately, these researches could not be proceeded with.

It may here be mentioned that attempts to produce immune bodies in the rabbit by inoculations of various cultures of *B. welchii* were, as has been noted by other authors, quite unsuccessful. Neither agglutinins nor amboceptor, as sought for by complement deviation tests, were found after repeated inoculations of doses covering a considerable range of both living and dead cultures. On the other hand, good agglutinating sera were easily prepared against *B. butyricus*, *B. tertius*, *B. cochlearis*, and *B. sphenoides*; but again circumstances intervened which prevented this portion of the research being proceeded with. The results obtained in some of these experiments are given in the following tables:

Table VIII.

Agglutination of various strains of B. sporogenes and other organisms with serum of rabbits immunized with the more common subterminal sporing type and the less common terminal sporing type of B. sporogenes.

Strain.	*Sporing type.*	*Result of agglutinating with serum of rabbit immunized by :*	
		Subterminal sporing type.	*End-sporing type.*
B. sporogenes.			
Forrest I	Subterminal	+	o
Boone II	„	o	o
Johnson	„	+	o
Pretty	„	+	o
Cullingford I	„	+	o
McFadzean II	„	+	o
„ IV	Terminal	o	+
Harris IV	„	o	+
Dawson	„	+	+
Ingram II	„	+	o
Parker A	Subterminal	o	o
„ B	„	+	o
„ C	„	+	o
McArdle I	„	+	o
„ III	„	o	o
Other organisms :			
McFadzean I		o	o
B. tertius		o	o
Cullingford III *b*		o	o
„ III *c*		o	o
„ X		o	o

Table IX.

Showing that from the same patient there were isolated several different members of the putrefactive group of bacteria which seemed quite distinct serologically.

Strain name.	*Strain no.*	*Type.*	*Sporogenes serum.*		
			Subterminal sporing type.	*End-sporing type.*	*Cullingford X serum.*
Cullingford	I	*B. sporogenes* subterminal spore	+	o	o
	III *b*	?	o	o	o
	III *c*	?	o	o	o
	X	?	o	o	+
McFadzean	I	?	o	o	o
	II	*B. sporogenes* subterminal spore	+	o	o
	IV	„ terminal „	o	+	o
Parker	A	*B. sporogenes* subterminal spore (colonies grew down into agar).	o	o	o
	B	*B. sporogenes* subterminal spore	+	o	o
McArdle	I	*B. sporogenes* subterminal spore	+	o	o
	III	„ „ „	o	o	o

(ii) *An unnamed bacillus isolated from one case only, known as Cullingford III c.*

This bacillus was isolated from a glucose agar plate made from pus which had been heated to 65° C. taken on the patient's admission to hospital.

Morphology. The bacillus is of medium size, straight or slightly curved, with square ends. After being incubated for 24 hours there is marked spore formation, the spores being oval and resembling those of *B. sporogenes* in size and shape. A very marked characteristic was the rapidity with which the body of the bacillus disintegrated after the spore had formed, even after 24 hours' incubation practically all the spores were lying free. In specimens made from very young cultures Gram's stain was positive, but this property was very soon lost, even a 24-hour-old culture showing but few Gram-positive elements. No motility could be made out even in the youngest cultures.

Cultural characters. The colonies on agar were irregular with a wavy edge from which projected a few filamentous processes. They were less opaque than those of *B. sporogenes*, and the processes were less numerous and less marked. Cultures in broth showed in 24 hours an even clouding of the medium. In older cultures the bacteria had settled to the bottom, leaving the supernatant broth clear. On gelatine incubated at 20° C. no growth could be made out. Milk cultures remained unchanged for the first 12 days. After 14 days' incubation, clotting had occurred. The clot was very slowly digested, the digestion not being complete after 6 weeks' incubation. Throughout this whole period the reaction remained alkaline. On red egg, after 24 hours' incubation the colonies appeared as round depressions on a pink field, after 48 hours the depressions had increased in size and the surrounding medium had become bright red. After 14 days there was distinct liquefaction of the coagulated egg but even at the end of 6 weeks much of the medium remained solid. In this stage the culture gave off a very putrid smell. In broth containing pieces of meat, growth was rapid and abundant, much gas being formed. No blackening of the meat was observed nor was any digestion noticeable to the naked eye. The smell of these cultures was intensely putrid.

Fermentation reactions. When grown on broths containing various sugars, &c., it was found that glucose and glycerine were fermented, both acid and gas being produced, while it was without action on all the other carbohydrates which were employed.

(iii) *An unnamed bacillus isolated from one case only, known as Cullingford III b.*

Morphology. This bacillus was rather small with rounded ends and frequently occurred in pairs. It stained readily and very sharply with the usual basic stains and retained Gram's stain. Examination of young cultures showed very distinct motility, the progressive movements having a somewhat sinuous character. Spore formation was not very abundant, the individual spores being

rather smaller than those of *B. sporogenes* ; as regards their position in the body of the bacillus they were either central or subterminal. The body of the bacillus rapidly degenerated after the spore had formed so that most of the spores were seen lying free.

Cultural characters. The colonies on agar were small, semi-transparent, round or slightly irregular. No filamentous processes were to be seen unless the plate was very wet, when there was formed a sheet of growth advancing with an irregular edge. Cultures in broth showed an even turbidity. Gelatine stab cultures incubated at 20° C. were rapidly liquefied and considerable amounts of gas were produced so that the medium was broken up. With milk cultures which had been incubated at 37° C. for 7 days the milk showed no change. At the end of 12 days it appeared to be somewhat digested, but no clotting could be made out. When, however,

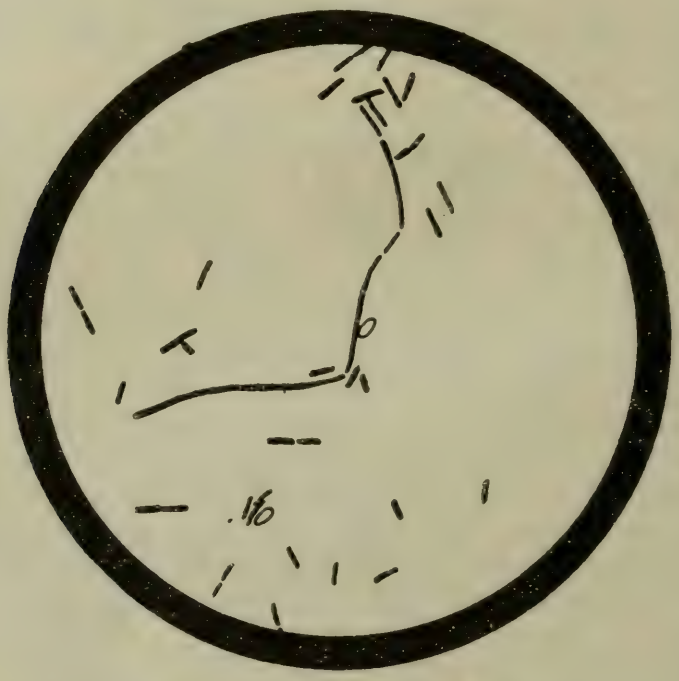


Fig. XIV. Anaerobic bacillus known as Cullingford III *b*.

the cultures were examined after 14 days' incubation a definite clot was found to have formed. The digestive process continued very slowly so that after 6 weeks' incubation some of the clot still remained undigested. Cultures on red egg showed, after 48 hours' incubation, colonies of the 'medusa head' type on a red background. After 8 days' incubation these colonies had formed yellow depressions. Immediately around them the medium was intensely blackened, the blackening being much more pronounced than in cultures of *B. sporogenes*. The remainder of the surface of the medium was a deep red. After 14 days the coagulated egg was markedly digested and in 6 weeks the liquefaction was complete and all the blackening had disappeared. The cultures gave off a strong smell of sulphuretted hydrogen in addition to the more usual smell of putrefaction. In broth cultures containing pieces of meat the blackening of the meat was very marked, and here again the smell of sulphuretted hydrogen could be recognized in addition to the smell of putrefaction. Spore

formation was marked, most of the spores lying free, but many were made out to be subterminal in position in respect to the body of the bacillus.

Fermentation reactions. In broths containing the various sugars, &c., it was found that glucose was fermented, both acid and gas being formed. In the case of glycerine, gas was produced, but the media apparently remained alkaline; there was no action on any of the other carbohydrates that were tested.

The main points of difference between this bacillus and *B. sporogenes* were as follows:

(1) In size this bacillus was smaller and spore formation was much less abundant. The individual spores even in young cultures had usually lost all signs of the body of the bacillus.

(2) Cultures on coagulated egg differed from those of *B. sporogenes* in that the digestion of the medium was very much slower, the blackening around the colonies was very much more intense, and the general appearance of young cultures was quite different.

(3) Emulsions of this bacillus were not agglutinated by any of the sera which agglutinated emulsions of *B. sporogenes*.

It may here be noted that from the same patient a strain of *B. sporogenes* was isolated which gave all the typical characteristics of that bacillus and which was agglutinated by standard anti-sera.

(iv) *An unnamed bacillus isolated from one case only, known as Cullingford X.*

Morphology. This was a large square-ended bacillus, it was *not* motile, Gram's stain was positive. Spore formation was very profuse and even in very young cultures fully formed spores were seen. The individual spores were oval, the long diameter being greatly in excess of the short; the body of the bacillus was not distended at the site of the formation of the spore. After the spore had been formed the body of the bacillus rapidly disintegrated, so that even in cultures only 24 hours old many free spores were seen.

Films made from cultures which had been incubated for 48 hours or more showed practically nothing but free spores, the bodies of the bacillus being represented by some ill-staining débris.

Cultural characters. The colonies on agar when growing on the surface were rather large, granular in structure, with somewhat irregular edges. When growing in the depths they appeared as opaque white spots from the surface of which passed fine tangled processes giving the colony a very woolly appearance. In broth the growth is very profuse and gives rise to an even clouding of the medium. On gelatine incubated at 20° C. no growth occurred. In milk after 7 days' incubation a clot had formed and much digestion had taken place. The reaction remained alkaline. The digestive process continued slowly and was almost complete after being incubated for 6 weeks. On red egg after 24 hours' incubation the colonies appeared as dirty yellow spots on a red background; after 48 hours' growth some liquefaction of the coagulated egg had commenced. Later the medium was noticed to be slightly blackened in the neighbourhood of the colonies. The digestion and lique-

faction of the medium continued very slowly and was not complete even at the end of 6 weeks' growth; the blackening of the medium persisted. In fluid cultures containing pieces of meat, blackening of the upper layers occurred after 24 hours' growth. The putrid smell was much less marked than in the case of *B. sporogenes.* Digestion of the meat proceeded very slowly.

Fermentation reactions. Glucose was fermented with the production of acid and gas, none of the other carbohydrates that were tested being affected.

A serum capable of agglutinating emulsions of this bacillus was obtained by injecting a rabbit with one dose of 500 million dead bacilli subcutaneously and three of 1,000 million intravenously.



FIG. XV. Anaerobic bacillus known as Cullingford X.

(v) *An unnamed bacillus isolated from one case only, known as McFadzean I.*

Morphology. This bacillus was rather short and stout, straight with rounded ends; young cultures showed it to be slightly motile. Gram's stain was positive. Spore formation was not very profuse. The spores were formed about the centre of the body of the bacillus, which disintegrated so rapidly after the spore was fully developed that it was rare to find a fully developed spore with the body of the bacillus capable of being stained.

Cultural characteristics. On agar plates the colonies were flat, irregular, and very transparent; there was a great tendency for the colonies to spread and to cover the whole surface of the medium with a thin film of growth. In broth culture growth appeared as an even clouding of the media. On red egg after 48 hours' incubation the colonies appeared as yellow depressions on a slightly reddish background. After a week's incubation there was considerable liquefaction of the egg but no blackening of the medium was ever noticed.

After one month's incubation some of the egg still remained solid as if the liquefying process had ceased. A strong odour of putrefaction was given off. In gelatine cultures incubated at 20° C. no growth occurred.

Fermentation reactions. Practically the only indication that any of the carbohydrates tested were acted on was a slight change in the reaction of the medium. Thus cultures in broths containing saccharose, mannite, glucose, and lactose were found after 48 hours' incubation to have lost the alkalinity that was present and to have become neutral; in the case of salicin, however, the alkalinity was increased, while inulin, glycerine, dextrin, and starch remained unchanged

Milk cultures were clotted after six days' incubation, the clot being digested later; the reaction remained alkaline.

(vi) *An unnamed bacillus isolated from one case only known as Hancock II.*

Morphology. This was a small bacillus about half the size of *B. welchii*; it was straight or very slightly curved with square ends. Gram's stain was positive and young cultures showed motility. Spore formation was not very profuse; the individual spores were oval and somewhat smaller than usual, the bodies of the bacilli not being distended at the point where they were formed. Their situation was usually subterminal but occasionally terminal.

Cultural characteristics. On agar or glucose agar surface colonies grew as flat very transparent structureless sheets varying in size from 0·5 mm. to 10 mm. in diameter. The margins were quite smooth and sharp-cut. In milk cultures, after 14 days' incubation the milk was clotted, later considerable digestion of the clot slowly occurred. In gelatine stab cultures at 20° C. there occurred liquefaction with slight gas formation. On red egg the growth after 48 hours' incubation appeared as a greasy looking slightly depressed scum on an orange background. The surrounding medium never took on a red tint which most of the other anaerobic bacilli produced. No blackening of the medium was ever noticed. Later liquefaction of the coagulated albumen slowly progressed until the whole mass was liquefied, but this occurred very much more slowly than in the case of *B. sporogenes.*

Fermentation reactions. Glucose was fermented with the production of acid and a small quantity of gas, none of the other carbohydrates which were tested being affected.

(*f*) Bacteria that cannot be placed in any of these Groups.

(i) *A bacillus isolated from one case only, known as Boone I.*

This bacillus was obtained from pus which had been heated to 65° C. for half an hour on the day that the patient was admitted.

Morphology. A specimen made from an agar culture 24 hours old showed a large, stout, square-ended bacillus identical in appearance to *B. welchii.* No spores were observed. In specimens made

from cultures grown on red egg the bacilli were seen to be much longer and more slender than those taken from agar cultures—this is exactly the opposite to what is seen in similar cultures of *B. welchii*. Specimens made from a ten-day-old culture on red egg showed a long and comparatively thin bacillus with many spores which were oval in shape and situated terminally.

Cultural characteristics. This bacillus was not a very strict anaerobe, in this character it was similar to *B. welchii*. On agar the colonies were round, semi-opaque, flat and showed a number of concentric rings on their surfaces. In size they were rather small, after 48 hours' incubation varying from the size of a pin point to 2 mm. in diameter. The colonies were well separated. In broth growth was rapid and profuse so that in 24 hours a thick, even



Fig. XVI. Anaerobic bacillus known as Boone I.

cloudiness of the medium was produced. In agar stabs the growth along the track of the needle was rather irregular ; it occurred to within 2 mm. of the surface. In gelatine stab cultures incubated at 20° C. irregular growth occurred along the needle track, later the gelatine was liquefied and some gas was formed.

On red egg after 36 hours' incubation the colonies showed as yellow spots on a bright red background. They formed slight depressions in the surface of the medium which at first were quite sharply defined. After one month's incubation the colonies had spread considerably and tended to become confluent. There was no further sign of digestion of the coagulated albumen other than the slight but constant depression which formed in the immediate neighbourhood of the colony within the first 48 hours.

Milk cultures showed no change in 48 hours. After 8 days' incubation a definite solid clot was formed which then showed no tendency to contract. After one month's incubation the clot had

contracted considerably and there appeared to be slight digestion. The milk remained alkaline throughout the whole period.

Fermentation reactions. Glucose was fermented, acid and gas being formed. In glycerine broth slight acid formation was observed after 15 hours' incubation, but in 48 hours the reaction had again become definitely alkaline; some gas had also been formed in 48 hours. The remaining carbohydrates which were tested remained unaffected.

Serum. An agglutinating serum with a titre of 1 in 3,200 was obtained by inoculating a rabbit with one dose of 500 million dead bacilli subcutaneously and two doses of 1,000 million intravenously.

This bacillus, although, when grown on agar, it closely resembled *B. welchii* in morphology, was more nearly related to *B. sporogenes* in its cultural characters; further it had the power of slightly digesting coagulated egg, it liquefied gelatine and had very limited power of splitting up the various carbohydrates.

(3) The Streptococci.

Very shortly after commencing to investigate the bacterial flora of the wounds it became evident that streptococci of some type were present in practically every septic wound and that these cocci were responsible for most of the complications that might occur during the healing of these wounds. It was therefore decided to investigate this group of cocci with rather more than usual care. In order to carry this out a large number of strains of streptococci were isolated from the samples of pus which were examined from day to day.

(*a*) Methods of Differentiation.

Each strain was put through a series of tests and the following characteristics were noted:

1. The naked eye characters of a 24-hour broth culture were noted to ascertain whether the growth consisted of an even turbidity throughout the medium or a granular deposit at the bottom of the tube, leaving the supernatant broth clear.

2. Microscopical preparations were made from a 24-hour-old broth culture, one of which was examined unstained. According to the length of the chains the cocci were classed as either 'longus' or 'brevis'. Another preparation was stained by Gram's method and counter-stained with neutral red to ascertain the shape, &c., of the individual cocci and the degree with which they retained the Gram's stain.

3. Fermentation tests. Cultures were made in broths containing lactose, saccharose, raffinose, mannite, and salicin, and the formation of acid was noted. (The medium used in these tests was made from casein as described by Cole and Onslow[1], the indicator being an alkaline solution of acid fuchsin.)

4. Cultures were made in litmus milk to ascertain if acid and clot were produced.

[1] Cole and Onslow, *Lancet*, 1916, ii. 9.

5. A culture was made in a sugar-free broth tinted with neutral red and incubated anaerobically for 48 hours ; it was then examined for the presence or absence of fluorescence.

6. A stab culture was made in gelatine and incubated at 20° C. ; after an interval of a week it was examined to ascertain if growth had occurred, and whether it had been accompanied by liquefaction of the gelatine.

When the data given by tests referred to above had been collected the various strains were arranged according to Gordon's modification of Andrewes's and Horder's classification.

Before the results thus obtained are described, attention may be drawn to the following points which were noticed during the isolation and testing of the various strains.

(*b*) Some General Considerations with regard to Streptococci.

1. Practically all the *Streptococci* found in wounds grow much more readily under anaerobic than aerobic conditions, and it was by no means uncommon to find a *Streptococcus* which later proved to be *Streptococcus pyogenes*, that would, when first isolated, only grow under anaerobic conditions ; it was only after they had been subcultured on several occasions that growth of such cocci under aerobic conditions could be obtained.

The importance of this observation cannot be over-estimated. In a case of rat-bite fever, for instance, the present authors [1] obtained the growth of a *Streptococcus* in 3 out of 5 cultures, incubated under anaerobic conditions, made from a lymphatic gland removed from the patient's axilla, whereas 5 other cultures which were incubated aerobically all failed to give any growth. This *Streptococcus* when first isolated gave no surface growth either in agar stab cultures or on agar slopes under aerobic conditions, but after being repeatedly subcultured it grew well under these conditions ; if none of the cultures had been incubated anaerobically an important piece of evidence pointing to *Streptococcus pyogenes* being the cause of the disease in this case would have been lost.

Again, *Streptococcus pyogenes* was isolated on three different occasions from one of the patients (Case No. 26) of the present series, but none of the primary cultures nor even the first few subcultures would grow under aerobic conditions. It was only after several stab cultures in agar had been made that surface growth could be obtained.

2. Cultures of most of the varieties of *Streptococci*, made on Dorset's egg medium or coagulated serum, remain alive for a much longer period than those on agar and gelatine.[2] The viability, however, of the different types was very variable. Some cultures died out unless subcultured every few days, but usually those made on coagulated egg or serum were alive and could be subcultured

[1] Douglas, Fleming, and Colebrook, *Lancet*, 1918, i. 253.

[2] More recently we have found that minced meat medium such as is used for the cultivation of anaerobic spore-bearing bacilli has the same function of preserving the vitality of *Streptococcus* cultures and indeed in this medium almost all the common microbes can live for long periods without subculture.

without any difficulty three months after being planted. They were kept at room temperature and the media prevented from drying up by a rubber cap fitted over the wool plug.

(*c*) Classification of the Strains of Streptococci isolated from 30 Cases taken at random.

In all 160 strains were isolated from these 30 cases and, using the classification mentioned above, the following results were obtained :

i. *Streptococcus pyogenes* .	. .	118 = 72·75	per cent.
ii. *Streptococcus salivarius*	. .	10 = 6·25	,,
iii. *Streptococcus faecalis* .	. .	8 = 5	,,
iv. *Streptococcus equinus* .	. .	5 = 3·125	,,
v. *Streptococcus anginosus*	. .	2 = 1·25	,,
vi. Unclassified *Streptococci*	. .	17 = 10·625	,,
	Total	160	

From 28 out of the 30 cases *Streptococcus pyogenes* was isolated on one occasion at least and in 15 out of the 30 cases it was the only *Streptococcus* found.

The 10 strains of *Streptococcus salivarius* were isolated from 3 different cases and were always associated with other streptococci, these being *Streptococcus pyogenes* in all 3 cases and *Streptococcus equinus* in 2 cases.

Streptococcus faecalis was isolated from 5 cases ; in all of these they were associated with other streptococci, in 4 instances with *Streptococcus pyogenes*, and in 1 with *Streptococcus equinus* and unclassified *Streptococci*.

The 5 strains of *Streptococcus equinus* were isolated from 4 cases. In 2 cases they were associated with *Streptococcus pyogenes*, in 1 case with *Streptococcus faecalis* and unclassified *Streptococci*, and in 1 with unclassified *Streptococci* only.

The 2 strains of *Streptococcus anginosus* were both isolated from the same case, they were associated with *Streptococcus pyogenes*.

The 17 unclassified *Streptococci* were isolated from 7 cases, in 5 cases they were associated with *Streptococcus pyogenes*, in 1 case with *Streptococcus faecalis* and *Streptococcus equinus* and in another case with *Streptococcus equinus* only.

(*d*) The Characters of the Various Types of Streptococci.

(i) *Streptococcus pyogenes.*

Morphology. Microscopical preparations made from a 24-hour-old broth culture show the microbes to be arranged in long chains and the individual cocci to be round and sharply cut, very few irregular or involution forms being seen. Gram's stain is well retained. Broth cultures after 24 hours' incubation show a well-marked granular deposit and often some granules sticking to the sides of the culture tube, but the main mass of the broth remains quite clear.

Fermentation reactions. The majority of strains ferment lactose,

saccharose, and salicin, with the production of acid, leaving raffinose and mannite unaffected. Occasionally, however, mannite is also acted on. Strains which ferment mannite appear to always retain this property. Some strains produce acid and clot in milk, others appear to have no action, no clot being produced, and the reaction remains alkaline ; others again produce only a slight amount of acid, but no clot.[1] Cultures in broth containing neutral red when incubated anaerobically did not as a rule show fluorescence. Gelatine cultures incubated at 20° C. showed good growth ; there was no liquefaction of the medium.

As was mentioned above this coccus always grows better anaerobically, and some cultures when first isolated will only grow under anaerobic conditions.

The pathogenicity of the various strains was not tested.

Taking the whole series of the 118 strains of *Streptococcus pyogenes* that were isolated and tested, the impression acquired was that this group of micro-organism forms a constant and compact group with very constant distinguishing characteristics, the only important variable factor being the power of some strains to ferment mannite.

In order to confirm this impression it was decided to carry out a series of agglutination tests, and to ascertain if a serum prepared by inoculating a rabbit with a series of doses of a vaccine made from one strain would agglutinate emulsions of this strain, and also the other strains, to approximately the same titre.

A vaccine was therefore made from a *Streptococcus pyogenes* isolated from Case No. 20 by heating a serum broth culture to 60° C. for 30 minutes. This vaccine contained 8,000 million cocci per c.c. and as a primary dose 1,000 million cocci were given subcutaneously ; four or five days later 1,500 million cocci were inoculated intravenously and three further doses of 8,000 million cocci were administered at intervals of one week. The rabbit was bled nine days after the last dose. The serum thus obtained was found to agglutinate an emulsion of the *Streptococcus* from which the vaccine had been made in dilutions up to 1 in 500.

The technique employed in carrying out these agglutination tests was to mix 1 c.c. of the dilution of the serum with 1 c.c. of a faintly opalescent suspension of the strain of the *Streptococcus* to be tested in small test tubes which were then placed in a water bath at 45° C., and the result was noted four hours later. The dilutions of the serum that were used were 1 in 50, 100, 150, 200, 300, 400, 500, 600, 800. The emulsion was prepared as follows : the strain of *Streptococcus* to be tested was planted in a tube containing about 5 c.c. of a mixture of trypsinized meat broth and serum or hydrocele fluid, the latter having been heated to 60° C. for 30 minutes. The proportion of serum to broth was about one-half to one-third. The tubes were then incubated at 37° C. for from 24 to 36 hours, preferably with the tube in a sloped position,

[1] Fleming and Porteous have since shown (*Lancet*, 1919, ii. 49) that the clotting of milk by *Streptococcus pyogenes* is largely dependent on the size of the tube in which the test is carried out. In very narrow tubes clotting is more rapid than in wide tubes, and in narrow tubes clotting may be observed when even after prolonged incubation no change can be seen in the wide tubes.

as a greater quantity of growth occurred when this was done. In this mixture all the strains of *Streptococcus pyogenes* were found to grow in such short chains that in shaking up the culture an almost even turbidity was produced throughout the medium. After being thoroughly shaken, the cultures were diluted to a faint opacity with salt solution.

Twenty-four strains of *Streptococcus pyogenes* which had been isolated from different patients were tested in this way. The results are given in the following table:

TABLE X.

No. of case from whom the streptococcus was isolated.	*Dilutions of Serum.*									
	1 in 50.	*1 in 100.*	*1 in 150.*	*1 in 200.*	*1 in 300.*	*1 in 400.*	*1 in 500.*	*1 in 600.*	*1 in 800.*	*Control.*
No. 2 ..	+	+	+	+	+	+	+	−	−	−
„ 5 ..	+	+	+	+	+	+	+	−	−	−
„ 7 ..	+	+	+	+	+	+	+	+	−	−
„ 8 ..	+	+	+	+	+	+	+	+	−	−
„ 9 ..	+	+	+	+	+	+	+	+	−	−
„ 10 ..	+	+	+	+	+	+	+	+	−	−
„ 11 ..	+	+	+	+	+	+	+	+	−	−
„ 12 ..	+	+	+	+	+	+	+	+	−	−
„ 14 ..	+	+	+	+	+	+	+	+	−	−
„ 15 ..	+	+	+	+	+	+	+	+	−	−
„ 16 ..	+	+	+	+	+	+	−	−	−	−
„ 18 ..	+	+	+	+	+	+	+	+	−	−
„ 19 ..	+	+	+	+	+	+	+	+	−	−
„ 20 ..	+	+	+	+	+	+	+	−	−	−
„ 21 ..	+	+	+	+	+	+	+	−	−	−
„ 23 ..	+	+	+	+	+	+	+	+	−	−
„ 24 ..	+	+	+	+	+	+	+	−	−	−
„ 26 ..	+	+	+	+	+	+	+	−	−	−
„ 27 ..	+	+	+	+	+	+	+	−	−	−
„ 29 ..	+	+	+	+	+	+	+	−	−	−
„ 30 ..	+	+	+	+	+	+	+	+	−	−
„ 31 ..	+	+	+	+	+	+	+	+	−	−
„ 33 ..	+	+	+	+	+	+	+	+	−	−
— A.B. ..	+	+	+	+	+	+	+	+	−	−

The strain from the vaccine used in inoculating the rabbit was isolated from Case No. 20.
The sign + indicates that agglutination was present with that dilution of serum.
The sign — indicates that no agglutination took place.

The following experiment was then made to ascertain if the various strains of *Streptococcus pyogenes* isolated from the same case were agglutinated by the immune serum to the same titre. Emulsions were prepared by the method described above from 4 strains isolated from Case No. 5, 6 strains from Case No. 7, 2 strains from Case No. 10, and 5 strains from Case No. 11. These emulsions were then mixed with dilutions of the immune serum similar to those employed in the former experiment. An emulsion was also prepared from strain 1, Case No. 20, which furnished the vaccine with which the rabbit yielding the immune serum was inoculated, and the agglutination given by the serum with this emulsion gave the standard titre.

The results are shown in the following table:

TABLE XI.

Strain from which emulsion was made.	*Dilutions of Serum.*								
	1 in 50.	*1 in 100.*	*1 in 150.*	*1 in 200.*	*1 in 300.*	*1 in 400.*	*1 in 500.*	*1 in 600.*	*Control.*
Case No. 20 (1) ..	+	+	+	+	+	+	+	—	—
Case No. 5 (1) ..	+	+	+	+	+	+	+	—	—
(2) ..	+	+	+	+	+	+	+	—	—
(3) ..	+	+	+	+	+	+	+	—	—
(4) ..	+	+	+	+	+	+	+	—	—
Case No. 7 (1) ..	+	+	+	+	+	+	+	—	—
(2) ..	+	+	+	+	+	+	+	—	—
(3) ..	+	+	+	+	+	+	+	—	—
(4) ..	+	+	+	+	+	+	+	—	—
(5) ..	+	+	+	+	+	+	+	—	—
(6) ..	+	+	+	+	+	+	+	—	—
Case No. 10 (1) ..	+	+	+	+	+	+	+	—	—
(2) ..	+	+	+	+	+	+	+	—	—
Case No. 11 (1) ..	+	+	+	+	+	+	+	—	—
(2) ..	+	+	+	+	+	+	+	—	—
(3) ..	+	+	+	+	+	+	+	—	—
(4) ..	+	+	+	+	+	+	+	—	—
(5) ..	+	+	+	+	+	+	+	—	—

These experiments indicate that an immune serum prepared by inoculating a rabbit with a vaccine prepared from a single strain of *Streptococcus pyogenes* is capable of agglutinating not only emulsions of the strain from which the vaccine was prepared but all the strains that were classified by the characters given above under the heading of *Streptococcus pyogenes*. Two points, however, remained to be investigated—one being whether these agglutination tests were truly specific or whether they were due to co-agglutination; the other was whether the small group of *Streptococci* which fermented mannite, but were otherwise similar in every way to those which failed to do so, were agglutinated to an equal titre by sera prepared by inoculating animals with vaccine made from strains which did not ferment mannite.

The following experiments were made to ascertain if the serum prepared by inoculating a rabbit with a vaccine made from a typical non-mannite fermenting strain of *Streptococcus pyogenes* agglutinated to a corresponding titre emulsions of the mannite fermenting strains.

Emulsions were prepared from the non-mannite fermenting strain used in the preparation of the serum, Case No. 20, Strain 1, and from three different mannite fermenting strains isolated from Case No. 13. These emulsions were then mixed with dilutions of the serum—1 c.c. of the emulsion to 1 c.c. of the dilution of serum.

The results are given in the following table:

TABLE XII.

No. of Case and Strain from which emulsion was made.	*Mannite fermentation.*	*Dilutions of serum.*							
		1 in 50.	*1 in 100.*	*1 in 150.*	*1 in 200.*	*1 in 300.*	*1 in 400.*	*1 in 500.*	*Control.*
Case No. 20. Strain 1	—	+	+	+	+	+	+	+	—
,, ,, 13. ,, 2	+	+	+	+	+	+	+	trace	—
,, ,, ,, ,, 3	+	+	+	+	+	+	+	trace	—
,, ,, ,, ,, 5	+	+	+	+	+	+	+	+	—

Former experiments showed that a 1 in 500 dilution of the serum was the highest giving any agglutination with the emulsion of Strain I, Case No. 20.

In the next experiment the emulsions were prepared from five other strains of the mannite fermenting type isolated from three different cases and the same serum was used in the tests; here again a similar result was obtained, the serum agglutinating these emulsions in dilutions up to but not beyond 1 in 500.

In these experiments the tubes containing the dilutions of serum and emulsion had been placed in a water bath at 45° C. for at least 4 hours before the readings were made.

In the following experiment emulsions from a non-mannite fermenting strain, Case No. 16, Strain 1, and a mannite fermenting strain, Case No. 8, Strain 5, were employed and the readings were made after 2 hours in the water bath at 45° C.

The result is given in the following table:

TABLE XIII.

No. of Strain and Case furnishing the emulsion.	*Mannite fermentation.*	*Dilutions of serum.* 1 in 10.	1 in 50.	1 in 100.	1 in 150.	1 in 200.	*Control.*
Case No. 16. Strain 1	−	+	+	+	+	trace	−
„ „ 8. „ 5	+	+	+	+	+	trace	−

These experiments show conclusively that a serum prepared by inoculating an animal with a vaccine prepared from a typical non-mannite fermenting strain of *Streptococcus pyogenes* has the power of agglutinating emulsions of the mannite fermenting strains to an exactly similar titre as is the case with emulsions of the non-mannite fermenting strains.

The next experiment shows that a serum prepared from a strain of *Streptococcus pyogenes* has no power to agglutinate emulsions of *Streptococci* belonging to the *faecalis* group.

In carrying out this experiment emulsions were made from three different strains of *Streptococcus faecalis*, and also to act as a control from the strain of *Streptococcus pyogenes* used in the preparation of serum. These were then put up in small test tubes with the varying dilutions of serum and the results were recorded after incubation at 45° C. for at least 4 hours.

The following table shows that the serum had no power to agglutinate emulsion of *Streptococcus faecalis* even in dilutions of 1 in 50, while the emulsion of *Streptococcus pyogenes* was agglutinated in dilutions up to 1 in 500:

TABLE XIV.

No. of Strain and Case furnishing the emulsion.	*Type.*	*Dilutions of serum.* 1 in 50.	1 in 100.	1 in 150.	1 in 200.	1 in 300.	1 in 400.	1 in 500.	*Control.*
Case No. 1. Strain 6	*faecalis*	−	−	−	−	−	−	−	−
„ „ 20. „ 4	*faecalis*	−	−	−	−	−	−	−	−
„ „ 33. „ 4	*faecalis*	−	−	−	−	−	−	−	−
Control 1 Case No. 20. Strain 1	*pyogenes*	+	+	+	+	+	+	+	−

Lastly an experiment was made to ascertain if, after the serum had had its agglutinating properties removed by adding to it an emulsion of a typical *Streptococcus pyogenes*, it would be incapable of agglutinating emulsions of other typical strains of *Streptococcus pyogenes* and also emulsions of mannite fermenting strains.

The experiment was carried out in the following manner: an emulsion of Strain 1, Case No. 16, a typical non-mannite fermenting *Streptococcus pyogenes*, was obtained by centrifuging three 24-hour-old broth cultures of the coccus, pipetting off the supernatant broth, and emulsifying the resulting deposit in 1 c.c. of normal saline solution. This gave a very thick emulsion, to which was then added 1 c.c. of the serum which had been prepared from Strain 1, Case No. 20, and was that used in the various agglutination experiments described above. The mixture was then incubated at 45° C. for 4 hours to allow the emulsified cocci to absorb the agglutinating property present in the serum. Dilutions of the serum were then made and mixed with emulsions prepared (i) from the strain used in absorbing the serum, (ii) and (iii) from two other typical non-mannite fermenting strains of *Streptococcus pyogenes*, (iv) from a mannite fermenting strain of *Streptococcus pyogenes*. The small test tubes containing these mixtures were then placed in a water bath at 45° C. and the results which are given in the following table were recorded after an interval of 4 hours.

TABLE XV.

No. of Strain and Case furnishing the emulsion.	*Dilutions of the absorbed serum.*			
	1 in 20.	*1 in 50.*	*1 in 100.*	*Control.*
Strain I. Case No. 16 (typical pyogenes used to absorb serum)	+	—	—	—
Strain VIII. Case No. 7 (typical pyogenes)	+	—	—	—
Strain I. Case No. 32 (typical pyogenes)	+	—	—	—
Strain V. Case No. 8 (mannite fermenting type of pyogenes)	+	—	—	—

The serum had been shown by previous experiments to have the power of agglutinating emulsions of all these strains in dilutions up to 1 in 500. The results given above, therefore, show that the serum after being absorbed by an emulsion of a typical *Streptococcus pyogenes* lost the power of agglutinating not only emulsions made from that particular strain but those made from other typical strains of *Streptococcus pyogenes* and also mannite fermenting types. This indicates that all the Streptococci described under the heading of *Streptococcus pyogenes* belong to one serological group and that the agglutination results obtained in the former experiments were specific and not due to the presence of group agglutination.

These experiments indicate that the various groups of *Streptococci* can be identified by the use of a specific agglutinating serum. A few preliminary experiments, however, showed that the precautions

found necessary in using this method for other bacteria are equally important in the case of *Streptococci*.

For instance, it was found that the specific serum for *Streptococcus pyogenes* agglutinated certain strains more nearly related to the *pyogenes* group in comparatively low dilutions of the serum. It is, therefore, important that the exact titre of the serum should be estimated and only those cocci which are agglutinated to about full titre should be placed in the group from which the serum was prepared.

(ii) *Streptococcus salivarius.*

Ten strains of this *Streptococcus* were isolated out of the 160 strains especially studied, that is 6·25 per cent. These 10 strains were isolated from 3 different cases out of the 30 from which the 160 strains of *Streptococci* were isolated. The characters of the cocci were as follows :

Morphology. A microscopical preparation made from a 24-hour-old broth culture showed the individual cocci to be rather small and round in shape. Involution forms were rather more common than in the case of *Streptococcus pyogenes*. The chains of cocci were distinctly of the type ' brevis '. Gram's stain was retained much less strongly than in the case of *Streptococcus pyogenes*. To the naked eye 24-hour-old broth cultures showed an even turbidity without any marked deposit at the bottom of the tube.

Fermentation reactions. Broths containing lactose, saccharose, and raffinose were fermented, acid being produced ; those containing mannite and salicin were unaffected. Litmus milk was rapidly clotted with the production of acid. No fluorescence was produced by growing the coccus in broth containing neutral red under anaerobic conditions for 48 hours. No growth took place on gelatine slopes or stabs incubated at 20° C. Cultures on agar and even on coagulated albumen died out very rapidly in comparison with most of the other varieties.

All the strains isolated were exactly similar to one another, but it was considered that as only 10 strains had been isolated from 3 cases agglutination tests would fail to give reliable results as to the unity of the group. No experiments were made to ascertain its pathogenicity for animals, but from the persistence and the abundance of the cocci in two of the cases from which they were isolated it was thought that they probably played an important rôle in these two wounds. Both of these cases were treated with apparent benefit with a vaccine made from the coccus isolated from the wound.

(iii) *Streptococcus faecalis.*

Cocci having the characters of this group were isolated on eight occasions. The general characters of this group were as follows :

Morphology. Microscopical preparations made from a 24-hour-old broth culture showed oval cocci which were very regular in size and shape and frequently occurred as diplococci. Gram's stain was retained to a very marked degree. To the naked eye 24-hour-old

broth cultures gave a perfectly even turbidity with no tendency to form a deposit at the bottom of the tubes.

Fermentation reactions. Broths containing lactose, mannite, and salicin were in every case fermented with the formation of acid; in certain strains which will be referred to later in detail saccharose was similarly acted on. Litmus milk was rapidly clotted with the production of acid. When grown in broth containing neutral red under anaerobic conditions for 48 hours the media became markedly fluorescent. In gelatine stabs or slopes incubated at 20° C. growth was rapid and abundant.

Two strains, which rapidly fermented saccharose, also had the power of slowly liquefying not only gelatine, but also coagulated albumen. This action was, compared to that exerted by many bacteria, very slow, as is indicated by the appearance shown in Fig. XVII, which is reproduced from a photograph of a culture grown for 8 days at 20° C. It was, however, progressive, and if the cultures were kept for several weeks or months, gradually the whole medium was rendered liquid. This was also true of cultures on coagulated albumen, at first the only sign of liquefaction being the formation of a slight trough under the line of growth and some cracks in the surrounding medium; after some months, however, for instance, in a culture on coagulated egg kept for 6 months at room temperature, practically the whole mass of coagulated proteid was liquefied into a grumous mass, brownish in colour.

(iv) *Streptococcus equinus.*

Five strains were isolated out of the 160 strains=5·125 per cent., and these were found in the wounds of 4 different patients.

The characters of this group of *Streptococci* were as follows:

Morphology. In microscopical preparations made from a 24-hour-old broth culture the individual cocci were seen to be round and regular in shape. The chains were always of the type 'brevis'. Gram's stain was retained less intensely than was the case with *Streptococcus pyogenes.* The naked eye appearance of a 24-hour-old broth culture was similar to that of *Streptococcus faecalis*, there being even turbidity throughout the medium and little or no tendency to form a deposit at the bottom of the tube.

Fermentation reactions. Broths containing saccharose and salicin were fermented with the production of acid; lactose, raffinose, and mannite were unaffected. Litmus milk usually remained unchanged. Broth cultures containing neutral red showed no fluorescence after being incubated under anaerobic conditions for 48 hours. Gelatine cultures incubated at 20° C. showed good growth.

No experiments were made to test the pathogenicity of this coccus, and from the fact that it was always isolated in the early stages of the wound and that it was never found on a number of different occasions from the same wound, its presence was thought to be most probably without pathogenic significance.

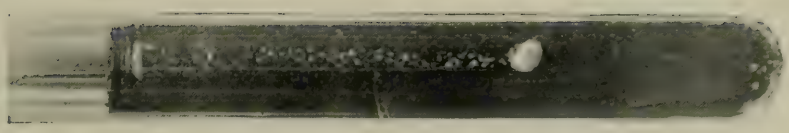


Fig. XVII. Stab culture of *Streptococcus faecalis*, showing liquefaction of the gelatine.

(v) *Streptococcus anginosus.*

Only two strains were isolated—both from the same case.

The characters of these *Streptococci* were as follows :

Morphology. In microscopical preparations made from a 24-hour-old broth culture the individual cocci were round and regular in shape. The chains of cocci were of the type ' longus '. Gram's stain was only moderately well retained. Broth cultures 24 hours old, examined with the naked eye, showed a marked granular deposit at the bottom of the tube, the upper portions of the medium usually remained clear but also occasionally showed some cloudiness.

Fermentation reactions. Broths containing lactose and saccharose were fermented, acid being formed. Raffinose, mannite, and salicin were unaffected. In litmus milk acid and clot were produced. Cultures in broth containing neutral red were rendered fluorescent after being incubated for 48 hours under anaerobic conditions. Gelatine cultures incubated at 20° C. showed little or no growth.

(vi) *Unclassified Streptococci.*

Streptococci giving characters which made it impossible to place them under any of the above headings were 17 in number, that is 10·625 per cent. of the 160 strains investigated. The 17 strains were isolated from 7 different patients.

(*a*) Six of these strains which were isolated from four different patients formed a group with the characteristics given below. Three of them were obtained from blood cultures, on each occasion only one tube giving growth out of ten that were planted with the blood, it was, therefore, probable that the cocci were present only in very small numbers. In one of the above cases it was also isolated from the wound. The three cases were all treated with a vaccine made from the strain isolated from the individual case, but improvement as a result of this procedure was not marked.

Morphology. In microscopical preparations made from a broth culture 24 hours old the individual cocci were very small, regular and round in shape. Gram's stain was not retained very intensely. The chains were usually of the type ' brevis ', but this was not so marked as in the case of *Streptococcus faecalis* or *salivarius*. To the naked eye a 24-hour-old broth culture generally showed some turbidity of the whole bulk of the medium and some deposit at the bottom of the tube.

Fermentation reactions. Cultures made in broth containing saccharose were fermented, acid being formed ; lactose, raffinose, mannite, and salicin were quite unaffected. Grown in milk, neither acid nor clot were formed. Gelatine cultures incubated at 20° C. gave good growth. Cultivated under anaerobic conditions for 48 hours in broth containing neutral red fluorescence was produced.

When planted on coagulated egg cultures, if kept at room temperature and drying up of the medium is prevented, the cultures remain alive for as long as seven months. No experiments were performed to ascertain its virulence for animals ; as regards its pathogenic

action in the wound the impression gained was that its presence was not of great importance.

(*b*) The next group consisted of four strains which were isolated from two patients, and which had the following characteristics :

Morphology. Microscopical preparations made from 24-hour-old broth cultures showed the individual cocci to be usually round, but even in young cultures a certain number of irregularly shaped involution forms were found. The chains formed by the cocci were rather intermediate in length but more nearly approached the type ' brevis ' than ' longus '. Gram's stain was retained less strongly than in the case of *Streptococcus pyogenes* or *faecalis*. Examined with the naked eye the 24-hour-old broth culture showed that growth occurred as a granular deposit at the bottom of the tube, the supernatant broth remaining clear.

Fermentation reactions. Broth cultures containing lactose and salicin were fermented, acid being formed ; saccharose, raffinose, and mannite were quite unaffected. In milk cultures acid and clot were rapidly produced. Cultures made in broth containing neutral red and incubated under anaerobic conditions for 48 hours showed no fluorescence. Gelatine cultures incubated at 20° C. gave good growth.

No experiments were made on animals to ascertain if this *Streptococcus* was pathogenic, but in one of the cases, at least, it appeared to play an important part as regards the condition of the wound during a period in the healing process. This was Case No. 13 who had an extensive wound in the region of the right scapula. The wound after an initial period of severe sepsis had been progressing favourably, and during this period *Streptococcus pyogenes* had been found in moderate numbers. The wound, however, again became unhealthy looking and cultures made on several occasions showed the presence of very large numbers of the *Streptococcus* under discussion. The aspect of the wound was quite striking, the granulations being very flat, glazed, and rather dusky in colour. The skin round it was reddened and sore. A vaccine having been made from the *Streptococcus* isolated, small doses were administered ; the improvement was very striking and the wound rapidly became healthy, so that a short time afterwards a very successful skin-grafting operation was carried out.

(*c*) The remaining 10 strains all showed individual differences which prevented them being allotted to definite groups. A considerable proportion were isolated from pus which had been heated to 65° C. for 30 minutes. These heat-resisting strains differed among themselves with respect to their fermentation reactions. Their presence in recently inflicted wounds—from which they were usually recovered—is readily explained by the fact that they are commonly to be found in normal faeces. The impression was gained that the heat-resisting types had no pathogenic significance in connexion with the wounds.

(4) The Diphtheroid Bacilli.

(*a*) Definition.

All Gram-positive bacilli which showed the characteristic so-called 'Chinese letter' or palisade arrangement have been classed as 'Diphtheroid Bacilli'.

(*b*) Number of Wounds Infected.

These bacilli were found either in cultures or in films of the pus from 54 patients at some period of their stay in hospital. From the specimens taken at the time of the patients' admission into hospital diphtheroid bacilli were found in 32 cases. Generally speaking it may be said that these bacilli are less commonly found in recently inflicted wounds than in the later stages, when granulation and healing of the wound is taking place.

In many of the pus films examined, diphtheroid bacilli were to be seen in enormous numbers both inside and outside the pus cells. It was a common thing to see here and there a pus cell packed with them, just as in gonorrhoeal pus occasional leucocytes are seen to be filled with *Gonococci* while the majority contain none. When such a pus was cultivated in the ordinary way there was often a surprisingly small yield of diphtheroid bacilli; indeed frequently none grew although they had been seen in large numbers in microscopical specimens of the pus. It is possible that some of them were obligate anaerobes, and from one case (No. 24) such an anaerobic diphtheroid bacillus was recovered. Wright[1], and Fleming[2], have described under the name of the 'wisp' bacillus an anaerobic diphtheroid bacillus occurring frequently in the early stages of wound infections.

(*c*) Possible Sources of Infection.

Diphtheroid bacilli are commonly found on the human skin, and since many of them are notably serophytic organisms (i. e. are able to multiply in the unaltered serum), their presence in the wounds may well have been due to direct infection from the surrounding skin. Doubtless also they were at times passed on from case to case, but in the present series of cases it is unlikely that this was so to any marked extent; for, as will be shown later, diphtheroid bacilli differing markedly from the more ordinary types and showing the characters of true diphtheria bacilli were found in four cases. These bacilli were found in patients widely separated from each other in the wards, and there was no evidence of spread of this type of bacillus from any of these patients to others in the neighbourhood.

It is possible that in some cases the bacilli seen in pus films were in reality acne bacilli. This bacillus, which in pus presents a very definite diphtheroid arrangement, is one of the commonest inhabitants of the skin and, if it gained access to the wounds, would be missed in the routine examination by cultures examined after 24 or 48 hours as its colonies do not become visible, even on a specially

[1] Wright, *Brit. M. J.*, 1915, i. 629.
[2] Fleming, *Lancet*, 1915, ii. 638.

suitable medium, for three or four days. The possibility of some of the diphtheroids being acne bacilli did not occur to the authors until after the investigation was completed, so there was no opportunity of testing it.

(*d*) Methods of Isolation and Differentiation.

The bacilli were isolated by picking off single colonies from plate cultures of the pus grown either anaerobically and aerobically for 24 or 48 hours at 37° C. The cultures were maintained on agar, but at some period cultures were made on coagulated serum and egg medium so that the morphology could be studied and the presence or absence of Ernst Babes granules could be noted. For the fermentation tests Cole and Onslow's digested casein broth was used as a basis, with the addition of acid fuchsin as an indicator and 1 per cent. of the appropriate fermentable substance.

In a number of cases it was noticed that in cultures which were believed to be pure, there were colonies of very different sizes, some being very large like those of *Staphylococcus*, while others more resembled those of *Streptococcus pyogenes*. On a number of occasions these were picked off and thoroughly tested, but it was found that there was no difference in the appearance of the organisms composing the large and the small colonies ; and that subcultures made from each type gave rise to a mixture of large and small colonies.

(*e*) Characters of 61 Strains of Diphtheroid Bacilli.

The characters of 61 strains, isolated from 21 patients, are set forth in Table XVI, and it can be seen from this that the bacilli arrange themselves into fairly definite groups. By means of the fermentation reactions the bacilli were split into four groups, and by other reactions two subgroups were separated from them. The main characters of these groups and subgroups are set forth in Table XVII.

There were certain minor differences within the groups. Thus in Group I, one strain (Case No. 1, Strain 2) showed bacilli which were larger than usual and which took the strain very irregularly, in this way resembling the bacilli of Group II. Three strains also of Group I grew on agar in colonies resembling streptococcal colonies and not in the large opaque colonies which were usual. In Group II also one strain (Case No. 4, Strain 1) gave *Streptococcus*-like colonies. One strain of Group IV fermented lactose.

It is possible that Groups I and II are in reality the same and that the mannite fermentation is of little importance. The authors have shown that in the case of *Streptococcus pyogenes* some strains ferment mannite although serologically they are apparently identical with the non-mannite fermenting strains, and possibly the same holds true for the diphtheroid bacilli. Mannite is much less easily broken up by these bacilli than either glucose or saccharose, and the acid formation, in almost all cases, did not appear before the second or third day of incubation. Their fermentative characters were constant and remained unchanged in strains tested after three months' cultivation on artificial medium.

Table XVI.

(In this table 'A' indicates that acid was produced during the growth of the organism. 'O' indicates that the fermentable substance was unaffected.)

Case No.	No. of Strain.	Date of isolation.	Characters on agar.	Morphology.	Neisser bodies.	Glucose.	Lactose.	Saccharose.	Mannite.	Glycerin.	Dextrin.	Raffinose.	Salicin.	Inulin.	Litmus milk.
1.	1.		Large round raised white colony.	Large bacilli. Irregular staining.	Few.	A	O	A	O	O	O	O	O	O	O
	2.		Large round raised fawn coloured colony.		,,	A	O	A	O	O	O	O	O	O	O
	3.	20.8.16.	Large round raised umbilicated colony.	Short stout bacillus. Few long forms.	,,	A	O	A	O	O	O	O	O	O	O
	4.	,,	Small round white colony.	Short stout bacilli. Uniform staining.	,,	A	O	A	O	O	O	O	O	O	O
	5.	,,	Ditto.	Ditto.	,,	A	O	A	O	O	O	O	O	O	O
	6.	5.9.16.	Like *Streptococcus*.	Medium length dotted bacillus.	,,	A	O	A	A	O	O	O	O	O	O
2.	1.		Large round raised white colony.	Stout, short, very irregular in shape and staining.	None.	O	O	O	O	O	O	O	O	O	O
3.	1.		Small colony, like *Streptococcus*.	Short stout bacillus. Uniform staining.	Few.	A	O	A	O	O	O	O	O	O	O
	2.		Large round raised fawn coloured colony.	Short stout fusiform bacillus. Irregular staining.	,,	A	O	A	A	O	O	O	O	O	O
	3		Ditto.	Ditto.	,,	A	O	A	A	O	O	O	O	O	O
	4.	18.8.16.	Large round flat fawn coloured colony.		,,	A	O	A	O	O	O	O	O	O	O
	5.	,,	Large round raised fawn coloured colony.		,,	A	O	A	A	O	O	O	O	O	O
	6.	23.8.16.	Large round raised white coloured colony.		,,	A	O	A	O	O	O	O	O	O	O
	7.	18.9.16.	Ditto.	Short septate.	,,	A	O	A	A	O	O	O	O	O	O
4.	1.	5.9.16.	Like *Streptococcus*. Fawn coloured old culture.	Short septate.	None.	A	O	A	O	O	O	O	O	O	O
	2.	,,	Ditto.	Ditto.	Few.	A	O	A	O	O	O	O	O	O	O
	3.	22.8.16.	Large round raised white colony.	Very short.	,,	A	O	A	O	O	O	O	O	O	O
	4.	5.9.16.	Ditto.	Short septate. Parallel arrangement.	Many.	A	O	O	O	A	A	O	O	O	O
6.	1.		Ditto.	Long bacillus. Irregular staining.	Few.	A	O	A	A	O	O	O	O	O	O
	2.	5.9.16.	Large round raised white umbilicated colony.	Medium length. Even staining.	,,	A	O	O	O	A	A	O	O	O	O
	3.	,,	Medium-sized round colony surrounded by buds.	Long bacillus, spotted.											

Case No.	*No. of Strain.*	*Date of isolation.*	*Characters on agar.*	*Morphology.*	*Neisser bodies.*	*Glucose.*	*Lactose.*	*Saccharose.*	*Mannite.*	*Glycerin.*	*Dextrin.*	*Raffinose.*	*Salicin.*	*Inulin.*	*Litmus milk.*
	4.	5.9.16.	Medium-sized opaque round colony.	Long bacillus, spotted.	Few.	A	O	O	O	A	A	O	O	O	O
	5.	18.9.16.	Small round white opaque colony.	Short septate.	,,	A	O	A	O	O	O	O	O	O	O
8.	1.		Large round raised colony, brownish tint.	Stout bacilli. Fusiform. Some clubbed. Irregular staining.	,,	A	O	A	A	O	O	O	O	O	O
	2.		Ditto. Flatter than S. I.		,,	A	O	A	A	O	O	O	O	O	O
	3.		Large round colony. Little raised. Brownish tint.		,,	A	O	A	A	O	O	O	O	O	O
	4.		Fine colony with depression in centre.		,,	A	O	A	A	O	O	O	O	O	O
	5.		As 1. Dry appearance of surface.		,,	A	O	A	A	O	O	O	O	O	O
	6.		As 2. Ditto.		,,	A	O	A	A	O	O	O	O	O	O
9.	1.		Like fine *Streptococcus* growth.	Short stout bacilli. Uniform staining.	None.	O	O	O	O	O	O	O	O	O	O
10.	1.	5.9.16.	Like *Streptococcus* colony but slightly irregular. Agar in old culture becomes fawn coloured.	Short septate bacilli. Some clubbed forms.	Few.	A	O	A	O	O	O	O	O	O	O
	2.	,,	Large round flat colony.	Short stout bacilli. Deeply stained spot in centre.	None.	A	O	A	O	O	O	O	O	O	O
11.	1.		Large round fawn coloured colony.	Short stout fusiform bacilli. Staining irregular.	Few.	A	O	A	O	O	O	O	O	O	O
12.	1.		Large round white colony.	Short stout, very irregular bacilli. irregular staining	,,	A	O	O	O	A	A	O	O	O	O
13.	1		Large round raised white colony.	Short stout fusiform bacilli. Irregular staining.	,,	A	O	A	O	O	O	O	O	O	O
14.	1.		Large round white colonies. Agar becomes port wine colour.	Medium length bacilli. Staining irregular.	,,	A	O	A	O	O	O	O	O	O	O
	2.		Ditto.	Ditto.	,,	A	O	A	O	O	O	O	O	O	O
18.	1.		Ditto.	Ditto.	,,	A	O	A	O	O	O	O	O	O	O
	2.		Ditto.	Ditto.	,,	A	O	A	O	O	O	O	O	O	O
	3	5.9.16.	Small round raised white colony.	Short septate bacilli.	,,	A	O	A	O	O	O	O	O	O	O
	4.	,,	Medium sized round umbilicated colony.	Long bacilli. Irregular staining.	Very many.	A	O	O	O	A	A	O	O	O	O
	5.	,,	Small raised umbilicated colony.	Short stout bacilli. Deeply stained spot in centre.	None.	A	O	A	O	O	O	O	O	O	O

Case No.	No. of Strain.	Date of isolation.	Characters on agar.	Morphology.	Neisser bodies.	Glucose.	Lactose.	Saccharose.	Mannite.	Glycerin.	Dextrin.	Raffinose.	Salicin.	Inulin.	Litmus milk.
21.	1.	1.9.16.	Like *Streptococcus*.	Long slender bacilli. Staining irregular.	Many.	A	A	O	O	A	A	O	O	O	O
	2.	5.9.16.	Large round raised white colony.	Short septate bacilli.	Few.	A	O	A	O	O	O	O	O	O	O
24.	1.	25.9.16.	Large round raised white umbilicated colony.	Short septate bacilli.	„	A	O	A	O	O	O	O	O	O	O
25.	1.	„	Large round flat opaque colony.	Irregular bacilli. Many clubbed barred staining.	„	A	O	A	O	O	O	O	O	O	O
	2.	„	Ditto.	Ditto.	„	A	O	A	A	O	O	O	O	O	O
	3.	„	Ditto.	Ditto.	„	A	O	A	A	O	O	O	O	O	O
	4.	„	Large round raised umbilicated colony.	Short septate bacilli.	„	A	O	A	O	O	O	O	O	O	O
26.	1.	„	Ditto.	Ditto.	„	A	O	A	O	O	O	O	O	O	O
27.	1.	4.10.16.	Ditto.	Ditto.	„	A	O	A	O	O	O	O	O	O	O
28.	1.	6.10.16.	Ditto.	Ditto.	„	A	O	A	O	O	O	O	O	O	O
	2.	„	Ditto.	Ditto.	„	A	O	A	O	O	O	O	O	O	O
	3.	„	Large round raised white colony. Agar becomes port wine colour.	Many long and clubbed forms.	„	A	O	A	O	O	O	O	O	O	O
29.	1.	„	Large round white colony.	Short septate bacilli.	„	O	O	A	O	O	O	O	O	O	O
	2.	6.10.16.	*Streptococcus* like growth.	Stout bacilli. Uniform staining.	None.	A	O	O	O	O	O	O	O	O	O
31.	1.	„	Large round white colony.	Short septate bacilli.	Few.	A	O	A	O	O	O	O	O	O	O
	2.	5.10.16.	Ditto.	Ditto.	„	A	O	A	O	O	O	O	O	O	O
	3.	6.10.16.	Ditto.	Large barred bacillus.	„	A	O	A	A	O	O	O	O	O	O
	4.	5.10.16.	Ditto.	Short septate bacillus.	„	A	O	A	O	O	O	O	O	O	O
	5.	„	Ditto.	Large slender bacillus.	Very many	A	O	O	O	A	A	O	O	O	O

Table XVII.

Group No.	*No. of patients from whom isolated.*	*Characters of colony on agar.*	*Morphology.*	*Neisser bodies.*	*Fermentations.*	*Pathogenicity for guinea pigs*
I	16	Large round colony which may be heaped up and umbilicated. The colour varies from white to a light fawn colour.	Short, stout bacilli usually showing a clear band in the centre.	Few.	Glucose and Saccharose.	0
I a	4	As I. except that the culture medium[1] becomes reddish brown and ultimately port wine colour. This coloration of the medium only takes place in the presence of air. It commences under the upper part of the streak on an agar slope, and in a broth culture incubated vertically it begins on the surface.	As I.	As I.	As I.	0
II	7	As I.	Rather shorter and stouter bacilli. Usually fusiform in shape but in some specimens many clubbed and pyriform bacilli are to be seen. The bacilli usually take the stain very irregularly.	Few.	Glucose, Saccharose, and Mannite.	0
III	3	Small colony resembling a *Streptococcus* colony.	Short stout bacilli staining uniformly.	None.	None.	0
IV	4	Fairly large round opaque colony.	Long slender bacilli exactly resembling true diphtheria bacilli.	Very many.	Glucose, glycerin, and dextrin.	+ *Post mortem:* pleural effusion, enlargement and congestion of adrenals.
IV a	1	As IV	As IV.	As IV.	As IV.	0

[1] The agar employed was made from a trypsin-digest broth by the method suggested by one of us (S.R.D.); see *Lancet*, 1914, ii. 891.

Group I a seemed a very definite entity because of its marked coloration of the culture medium, but in all other respects it resembled Group I. It is to be noted that in several cases it occurred in wounds together with bacilli of Group I.

Group III appears to be identical with Hoffman's bacillus both in its morphology and in its inability to ferment any of the test substances.

Considerable interest attaches to Group IV, as the bacilli included in it appear to be true Klebs-Loeffler bacilli. Their extraordinary resemblance to true diphtheria bacilli was noted at once by an examination of a film of the original cultures isolated. In specimens made from young cultures on serum, Neisser's stain showed polar granules in almost all the bacilli, and when guinea-pigs were injected with 1 c.c. of a 24-hour broth culture they died with lesions identical with those which follow the injection of true diphtheria bacilli. One of the cultures resembling *B. diphtheriae* was non-virulent for guinea-pigs, as were all the members of Groups I, I a, and II which were tested. The patients from whom these cultures of true diphtheria bacilli were isolated showed no clinical signs of such an infection. There was nothing at all resembling a diphtheritic membrane, and there were no special constitutional disturbances.

(*f*) Incidence of the Different Types of Diphtheroid Bacilli.

Table XVIII shows the arrangement of all the strains tested into their different groups, and it will be seen from this that of the 21 cases from whom diphtheroid bacilli were isolated, bacilli of Group I were found in 16, Group I a in 4, Group II in 7, Group III in 3, Group IV in 4, and Group IV a in 1. In a very large number of cases the diphtheroid infection was a mixed one. In four of the cases no less than three types were found, and it is worthy of note that in each of the wounds harbouring virulent diphtheria bacilli there were found representatives of one or more of the other types.

Note. In September 1917 Fitzgerald and Robertson[1] reported that true diphtheria bacilli were found in the wounds of 40 out of 67 patients arriving in Toronto between May 20 and June 7, 1917. In some cases, but not in all, the wounds showed a characteristic diphtheritic membrane. The extraordinary prevalence of this infection was explained by the fact that one of the nurses in charge of these wounded men had a slight wound on the finger, from which *B. diphtheriae* was isolated. Fitzgerald and Robertson's report led to an investigation of the bacteria of the diphtheroid group in wounds in Canadian Hospitals in England, and the results of this investigation have been published by Adami and others.[2] Out of 306 cases investigated, bacilli were found in four which had the morphological and cultural characters of *B. diphtheriae* and of these two were found to be pathogenic to animals.

[1] Fitzgerald and Robertson, *J. Am. M. Ass.*, 1917, **69**, 791.
[2] Adami and others, *Bull. Can. Army Med. Corps*, June 1918.

Table XVIII.

No. of strains tested which fell into Groups.

Case No.	*I*	*I a*	*II.*	*III.*	*IV.*	*IV a.*
1	4	2	0	0	0	0
2	0	0	1	0	0	0
3	3	0	3	1	0	0
4	3	0	1	0	0	0
6	1	0	1	0	3	0
8	0	0	6	0	0	0
9	0	0	0	1	0	0
10	2	0	0	0	0	0
11	1	0	0	0	0	0
12	0	0	0	0	0	1
13	1	0	0	0	0	0
14	0	2	0	0	0	0
18	2	2	0	0	1	0
21	1	0	0	0	1	0
24	1	0	0	0	0	0
25	1	0	3	0	0	0
26	1	0	0	0	0	0
27	1	0	0	0	0	0
28	2	1	0	0	0	0
29	1	0	0	1	0	0
31	3	0	1	0	1	0
Total No. of cases in which the different types were found	16	4	7	3	4	1

The Canadian workers investigated the characters of the various diphtheroid organisms in the wounds and classed them into five groups by means of their fermentation reactions. The results are shown in the following table :

Table XIX.

Type.	*No.*	*Dextrose.*	*Lactose.*	*Saccharose.*	*Dextrin.*
Wound diphtheroid No. 1	6	A	A	A	A
„ „ „ 2	6	A	A	A	O
„ „ „ 3 (Xerosis type)	23	A	O	A	O
B. diphtheriae :					
Virulent	2	A	A	O	A
Non-virulent	2				
Hoffmann's bacillus	2	O	O	O	O

These tests were carried out in Hiss's serum water medium.

It is to be noted that the Canadian observers found that a considerable number of the strains had the power of fermenting lactose, whereas the present authors in their series found only one strain (a true diphtheria bacillus) to have this property. Reference to the older literature on the subject indicates that the fermentation characters of *B. diphtheriae* are not quite constant, and possibly the difference in the nature of the test media employed accounted for the difference in the results obtained. There seems no doubt, however, that the common diphtheroid bacillus found in war wounds in England is a bacillus of the xerosis type which ferments only glucose and saccharose and is non-pathogenic for laboratory animals.

(*g*) Significance of Infection with Diphtheroid Bacilli.

Diphtheroid bacilli in wounds have almost always been regarded lightly. They are practically never found alone, and as their direct

pathogenicity is slight they are overshadowed by the *Streptococci* which are almost invariably present. It has been shown, however, that they exercise a powerful symbiotic influence on some of the pathogenic bacteria (vide Douglas, Fleming, and Colebrook [1]), and in this connexion their presence in an infected wound may be of considerable importance.

(5) B. proteus.

(*a*) Number of Wounds Infected and Persistence of the Infection.

B. proteus was isolated from the wounds of 40 out of the 61 cases investigated, on some occasion during their stay in hospital. Unlike the *Streptococci*, however, it was often only found after the patient had been in England for a varying period; thus 17 out of the 40 cases failed to give cultures when examined immediately after their admission, but did so at varying intervals of from 1 to 59 days later. It is probable, therefore, that this bacillus is usually a hospital infection.

As regards the length of time that the infection persisted in the wounds, there was considerable variation. In those cases where the wound was well drained, involved only soft parts and was in a healthy state, the infection frequently disappeared in a few days, but when sloughs, or especially sequestra, were present, the infection lasted for months, the bacillus apparently finding a nidus quite inaccessible to the antibacterial properties of blood fluids and cells.

Wounds infected with *B. proteus* showed no very distinctive characters; frequently, however, the surface had a somewhat unhealthy appearance and any newly formed epithelium tended to break down.

(*b*) The Isolation of B. Proteus and its General Characters.

B. proteus, as its name implies, is very pleomorphic in its morphology. Specimens made from young broth or agar cultures showed the predominating form to be a short bacillus with rounded ends, but long thread-like forms are not uncommon. The bacillus is easily stained with any of the basic dyes but does not retain Gram's stain. It is actively motile and, when suitably stained, shows peritrichous flagellae.

Owing to the characteristic growth on agar being the formation of a spreading film, which usually, in 16 to 20 hours, covers the whole agar surface of the plate or tube, the isolation of *B. proteus* is peculiarly easy, and for the same reason it is very seldom that it escapes recognition when present in a sample of pus. All that is necessary for the isolation of pure cultures is to make a subculture from the film as far as possible from the track of the needle with which the plate or tube was inoculated, so as to avoid the chance of picking up other bacteria that may be growing below the film.

The appearance of the growth on agar is fairly characteristic, the most marked feature, as already mentioned, being the formation of a film which rapidly spreads over the whole surface of the medium. This film is usually quite uniform, very little if any thickening being present to show where the colonies commenced. Its edge is usually

[1] Douglas, Fleming, and Colebrook, *Lancet*, 1917, i. 604.

smooth but somewhat irregular, and slightly thinner than the central portions. In appearance it is iridescent, especially when an agar slope is examined with transmitted light, but so colourless that in plate cultures after 16 to 20 hours' incubation, when the whole agar surface is covered with a thin layer of growth, it is almost invisible, and it is only when a portion of the film is removed with a platinum needle that the growth becomes evident. In older cultures the film becomes much thicker and assumes a dirty whitish colour, and often the medium throughout its whole thickness becomes stained a dirty brownish tint. The consistence of the agar is also altered in old cultures, the jelly becoming less coherent so that the surface breaks easily under the platinum needle.

Broth Cultures. In ordinary broth growth is abundant, an even turbidity being rapidly formed. With age the density increases and a collar of growth collects round the tube at the surface of the broth, but usually no true veil of growth is formed over the surface. At the same time there is a considerable deposit of growth at the bottom of the tube, but the intervening broth remains cloudy. The medium may assume a dirty brownish tint. These cultures have a very disagreeable smell.

Gelatine Cultures. In stab cultures growth at 20° C. is rapid, a line following the track of the needle being visible in 24 to 30 hours. Liquefaction of the gelatine rapidly takes place, commencing almost as soon as the growth is visible at the surface of the medium immediately around the needle track. The liquefaction spreads rapidly over the surface and downwards along the track of the needle, and a large proportion of the gelatine is usually completely liquefied in seven to nine days. On gelatine plates, prepared with 3 to 5 per cent. gelatine, growth at 20° C. is rapid and a characteristic appearance is shown soon after liquefaction has commenced. The centres of the colonies appear like dewdrops lying in a depression of liquefied gelatine, while very fine branching threads of growth can be seen radiating into the still solid medium.

Milk. Growth on milk is rapid and abundant ; the casein is precipitated and digested, and if litmus has been added the colour is usually rapidly bleached. The culture at this stage usually shows a faint dirty brownish colour with some partially digested casein at the bottom of the tube and turbid dirty whey above. The reaction is often slightly acid in the very early stages, but the medium rapidly becomes increasingly alkaline.

Fermentation of sugars, &c. Fermentation tests for the identification of *B. proteus* are very unsatisfactory, as the bacillus has the power of bleaching almost all the indicators ordinarily used in such tests. Further, although the bacillus has the power of breaking down certain sugars, for instance glucose, with the production of acid and gas, it has a very marked property of attacking proteid and its derivatives and during the process liberating a considerable amount of alkali. In consequence a culture may, after showing an initial acidity with accompanying gas production due to the action of the bacillus on the sugar or alcohol, again become alkaline as the result of the breaking down of peptone, &c., with the production of an excess of alkaline products ; under these circumstances cultures may show gas production and, at the time of examination, a strongly alkaline

broth. It may be noted, however, that mannitol is unaffected by all strains of *B. proteus*.

One biochemical test useful in recognizing *B. proteus* consists of planting the organism into slightly acid or neutral urine and incubating the culture at 37° C. Growth takes place rapidly, and after 4 to 6 hours' incubation the urine is found to be strongly alkaline and free ammonia is given off. Indol was not produced by the strains of *B. proteus* obtained from wounds (Wolf).[1] When grown under anaerobic conditions in a mixture of serum and trypsin, large quantities of gas are produced. Wolf working with certain selected strains isolated from these wounds found that *B. proteus* had but comparatively feeble proteolytic properties and should not be placed among the putrefactive organisms.

(*c*) Serological Races of B. proteus.

A series of experiments were carried out to ascertain if *B. proteus* isolated from wounds consisted of a single or a number of serological races. Two agglutinating sera were prepared by inoculating two rabbits, each with a vaccine prepared from a single strain of *B. proteus*. The agglutinating titre of these two sera to emulsions of the homologous organism was found to be 1 in 64,000 in the one, and 1 in 8,000 in the other.

Thirteen strains were then taken, and, emulsions having been prepared by diluting down broth cultures to a suitable opacity with salt solution containing 1 in 1,000 formalin, agglutination tests were carried out, each emulsion being tested with both the above sera. The results showed that the more powerful serum agglutinated all the thirteen strains, which included the homologous bacillus, to practically the same titre, namely a dilution of 1 in 64,000, but the other serum only agglutinated two or three of the same strains to approximately the same titre (1 in 8,000) as an emulsion of its homologous strain.

The conclusion deduced from the above tests and a large number of absorption experiments was that certain strains of *B. proteus* contained in their structure a large number of antigenic components, while other strains only contained a very limited number, with the result that a serum prepared from a strain containing the large number of antigenic components would tend to agglutinate a large number of strains, while that made from a strain possessing but a limited number of components would have the power of agglutinating but a small number of strains.

(6) B. pyocyaneus.

(*a*) Number of Wounds Infected—and Variation of the Strains as Regards Chromogenic Power.

B. pyocyaneus was isolated from 31 of the 61 cases investigated, i. e. 50·8 per cent. The cultural, morphological, and most other characteristics are so well known that it is not proposed to discuss them. One point, however, was especially noted—namely, the variations in the chromogenic power of the different strains. This appears a constant property of an individual strain and showed

[1] Wolf, *J. Path. and Bacteriol.*, 1919, 22, 289.

very wide differences, the colour produced in agar cultures varying from a very pale green to a deep reddish black. Occasionally strains isolated from the same patient gave different depths of colour.

As regards the period of infection, this pursued a very similar course to that described in the section dealing with *B. proteus*, and the conclusion arrived at was that *B. pyocyaneus* showed a great tendency to be spread from patient to patient during their stay in hospital. In some cases the length of time the infection persisted was very short, in others, however, as with *B. proteus*, probably owing to the presence of sloughs or sequestra, it was much more prolonged.

(*b*) Pathogenicity.

At times the presence of the organism in the wound seemed to cause no change in the condition of the wound or the patient; at others the infection coincided with an increase in general symptoms, such as pyrexia, and a cessation of the healing process together with an unhealthy appearance in the wounds. In such cases the treatment with vaccines gave very satisfactory results (vide Part I, p. 14).

Owing to the difficulty of producing satisfactory agglutinating sera for *B. pyocyaneus* the question of the existence of a single or a variety of serological races was not investigated.

(7) Staphylococci.

These cocci were very commonly found in the wounds, but as they appear to differ in no way from the *Staphylococci* which flourish in the ordinary open wound or superficial ulcer, no detailed description of their characters is necessary.

Staphylococci were found in 41 wounds at the first examination after the patients' admission to hospital. The patients had been wounded at intervals varying from 4 to 65 days before their arrival in London. In 14 of the wounds *Staphylococci* were not discovered at the first examination, but were found subsequently during their stay in hospital. The interval between the admission of these patients and the discovery of *Staphylococcus* varied from 2 to 58 days. In 9 of the wounds no *Staphylococci* were found at any time.

It is clear therefore that infection with *Staphylococci* was extremely common in these wounds. The type of coccus varied, and although the '*aureus*' variety was the most common, cocci of the '*albus*' type were frequently found. *Staphylococci* giving the pale greyish colonies characteristic of *S. epidermidis* were only rarely found.

(8) Coliform bacilli.

In this group are classed all the bacilli, except *B. pyocyaneus* and *B. proteus*, which in their morphological characters and in their profuse growth on ordinary media, resembled *B. coli*. They did not liquefy gelatine.

Such bacilli were found in most of the wounds at some period, but they did not usually persist. They were so varied in their characters that their classification has not been attempted, but a brief description of each of them will be found in the bacteriological notes appended to the case report of the individual patient from whom it was isolated (Part I).

PART III

EXPERIMENTAL WORK BEARING UPON THE ACTION OF CERTAIN ANTISEPTICS IN INFECTED WOUNDS

(1) Introductory.

In determining by laboratory tests the usefulness of this or that antiseptic in the treatment of septic wounds many methods have been employed. The old method of determining the lethal action of the antiseptic on bacteria in a watery medium, while it is useful in other spheres, is valueless when it is desired to estimate the value of a bactericidal agent in a wound.

For the treatment of an infected wound an ideal antiseptic ought to possess, amongst others, the following properties :

1. It must be capable of killing bacteria in the discharges of a wound, i. e. in serous fluid containing many leucocytes.
2. It must be capable of penetrating into small diverticula such as exist in all septic wounds, and of destroying the microbes in such recesses.
3. It must have the power of penetration into the walls of the wound so as to kill the bacteria which may flourish there.
4. It must not exercise any local destructive action on the tissues or interfere with the physiological properties of the tissue fluids or cells.
5. It must not, in the concentration employed, exercise any general toxic effect on the individual.

Up to the present no antiseptic has been produced which approaches the ideal. All the antiseptics in common use have a much greater destructive effect on animal cells than they have on bacteria ; none of them are capable of penetrating into the walls of a wound and exercising any destructive action on the bacteria without at the same time killing the tissue cells ; and none of them can penetrate into small diverticula of the wound and kill the microbes in such situations.

It was with such a scheme of the properties for an ' ideal ' antiseptic constantly in mind that the following experiments were carried out.

(2) Experimental methods.

A large part of the work consisted of *in vivo* experiments conducted upon two cup-shaped wounds, which lent themselves particularly well to investigation since a measured quantity of fluid could be readily introduced into them, retained for a while, and then removed for examination.

These two wounds will hereafter be designated ' P ' and ' C '. Both were cup-shaped depressions leading down into the cancellous tissue of the tibia, and both resulted from a compound fracture of this bone. They were each capable of retaining about 2 cubic

centimetres of fluid, and, being old wounds of many weeks' standing, were lined with granulation tissue. In many respects, however, they differed from one another; the one 'P' had walls of granulation tissue, but the base was occupied by a large sequestrum of spongy bone. From this wound there was a copious discharge of pus containing about 1,000,000 leucocytes per c.mm., which was heavily infected with anaerobic spore-bearing bacilli, *Streptococci*, coliform bacilli (mostly *B. proteus*), and diphtheroid bacilli. The volume of the discharge varied considerably from day to day and sometimes was as copious as one c.c. per hour. The other wound 'C' was wholly lined with granulation tissue, and, except under the action of certain solutions which will be described later, the quantity of discharge was fairly constant, amounting on the average to 0·2 c.c. per hour. This wound was infected only by pyogenic cocci.

The following procedure—or some slight modification of it—was employed in working with these wounds: the wound cavity, having been completely emptied, was washed out with repeated changes of normal saline solution until the washings were quite clear. All fluid was then carefully removed. After an hour the total exudate into the cavity was measured by sucking it up into a graduated pipette—and was retained for examination. The quantity and character of this exudate served as a control observation. After again washing out the cavity with normal saline and emptying it, exactly 1 c.c. of the antiseptic under investigation was introduced. This was allowed to remain for 5 or 10 minutes and then removed for examination after carefully mixing the whole contents of the cavity by sucking into and blowing out of the pipette. The quantity of fluid so removed was noted as before.

The wound was now filled in a second time with 1 c.c. of the antiseptic solution which was left there for a longer period, say half an hour, and then again removed for examination. This procedure was repeated as often as was required.

A number of samples of the antiseptic solution after different periods of contact with the wound having in this way been collected, each was examined in order to ascertain the following points:

1. The number of living bacteria present.
2. Whether the antiseptic solution had diminished in strength while it was in the wound.
3. The number of leucocytes present, and their condition.
4. Whether there had been exudation of serous fluid into the cavity—or, on the other hand, absorption from it—while the antiseptic was in the wound.
5. What was the anti-tryptic value of the serous exudate?

Where other experimental methods were employed, they will be described separately in connexion with the results obtained from each.

(3) Experiments bearing upon the ability of antiseptic solutions to kill bacteria in the discharges from an infected wound.

An answer was first sought to the question—Does a marked reduction in the number of living bacteria actually occur when antiseptics are introduced into an infected wound?

The antiseptics chosen for this inquiry were Dakin's solution, eusol (hypochlorite solutions) and chloramine T, as being bactericidal agents of rapid action—and flavine, as representative of the more slowly acting agents. Each of these was instilled on different occasions into one of the wounds 'P' or 'C' as described above, and the bactericidal effect determined by enumerating the living organisms per c.c. of fluid present after varying periods of treatment with the antiseptic.

The enumeration of the bacteria was made as follows: immediately a sample had been pipetted out of the wound cavity 10 c.mm. were taken up into a capillary pipette and thoroughly mixed with 50 c.c. of agar which had been melted and allowed to cool to 45° C. This mixture was poured into a Petri dish and allowed to solidify. To avoid surface growths which were very apt to spread over the medium, a second layer of melted agar at 45° C. was now poured into the dish. The plates were then incubated for 48 hours, when the colonies were counted.

Experiments with Dakin's solution.[1] (1) The wound 'C' was washed out, a sample of the normal exudation collected after one hour, and the viable microbes in it enumerated. Dakin's solution was then placed in the wound, replaced by fresh solution 5 times within 75 minutes and then withdrawn. One hour and 2 hours later, samples of the exudate were taken from the wound and the living bacteria in these enumerated. The 3 samples gave practically the same number of colonies.

(2) The Dakin's solution was applied to the wound 4 times during four and a half hours and then withdrawn. A sample of the exudation occurring before the application gave 91 colonies—a similar sample of the exudate taken 1 hour after the antiseptic had been withdrawn gave 95 colonies.

Experiment with Eusol. This was conducted in a slightly different manner. The wound 'P' was filled in with eusol; 10 c.mm. samples were withdrawn at intervals of 5, 20, 30, and 60 minutes, diluted 1,000 fold in normal salt solution to avoid carrying over any appreciable amount of antiseptic, and the bacteria then enumerated as before. The result is shown in Table XX.

TABLE XX.

Time the Eusol remained in the wound.	*No. of colonies developing.*	*Calculated no. of colonies per c.c. of fluid in the wound.*
5 mins. . . .	2	200,000
20 ,, . . .	50	5,000,000
30 ,, . . .	40	4,000,000
60 ,, . . .	25	2,500,000

With regard to this result it will be seen in the section immediately following, that the hypochlorite concentration of eusol was reduced more than 90 per cent. after being in the wound 10 minutes. Therefore, the lower bacterial count obtained with the 30 and 60 minute samples is probably not attributable to any bactericidal effect of the antiseptic; it is much more likely that it was due to the destruction of bacteria by the leucocytes, which emigrate into the wound in

[1] Daufresne's modification.

great numbers as soon as the hypochlorite concentration is sufficiently lowered (vide p. 78, Expt. 1, and also p. 108).

Experiments with Chloramine T. (1) A 1 per cent. solution was placed in the wound ' P ' and samples taken for enumeration of the bacteria at intervals, as shown in the following Table XXI.

TABLE XXI.

Time the solution had remained in wound.	*Number of colonies developing on plates.*
10 minutes	No growth visible after 24 hours. Innumerable colonies after 4 days' incubation.
20 „	Ditto.
30 „	Innumerable colonies after 48 hours' incubation.
60 „	Ditto.

This experiment shows that the application of a 1 per cent. solution of chloramine T to a heavily infected wound caused no appreciable diminution in the number of living organisms present, whether that application was short or prolonged.

The delayed growth of the agar cultures was presumably due to a certain amount of uncombined antiseptic carried over in the sample of fluid used for the enumeration. It will be noted that, in the case of the samples taken from the wound after 30 and 60 minutes application of the drug, growth occurred much more rapidly than in the earlier samples ; and reference to Section 4 will show that the reason for this, viz. that the active principle of the antiseptic had, within 30 minutes, been exhausted by combination with the tissues and exudate of the wound.

It will be instructive to refer here to Fig. XVIII, which depicts a film preparation made from the same wound on another occasion after a 1 per cent. solution of chloramine T had been applied to it in four changes during one hour. When so applied the antiseptic may be said to have had its maximum opportunity of exciting a bactericidal effect and yet the preparation shows a great proportion of microbes—not appreciably less, in fact—than were present in the wound when untreated. And the result given in Table XXI makes it clear that the microbes seen in the film were chiefly living organisms. It may be stated that film preparations made when testing the bactericidal effect of Dakin's solution and eusol (vide p. 75) showed precisely similar appearances.

(2) With a 4 per cent. solution of chloramine T the following result was obtained :

TABLE XXII.

Source of sample tested.	*No. of colonies developing from 10 c.mm. samples.*	
Preliminary wash with normal saline . . .	872	(Surface film of *B. proteus*).
Chloramine solution after 5 minutes in the wound	4	
„ „ „ 10 „ „ „	5	
„ „ „ 20 „ „ „	30	(Surface film of *B. proteus*).
„ „ „ 30 „ „ „	79	„ „ „
„ „ „ 50 „ „ „	764	„ „ „
Wash with normal saline solution immediately after the withdrawal of chloramine solution . .	800	„ „ „

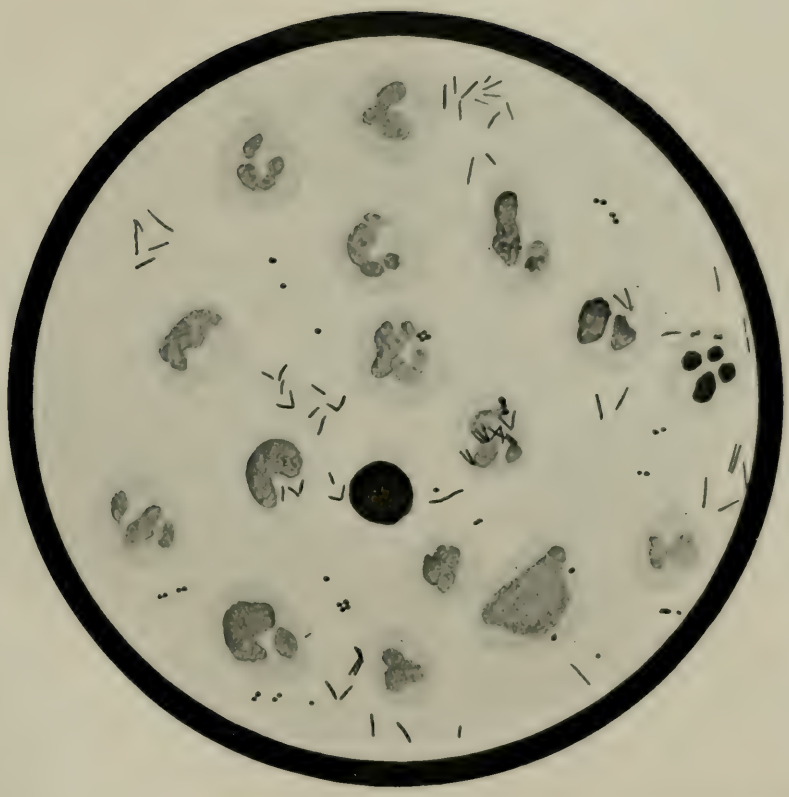


FIG. XVIII. A film preparation of the exudate from the same wound passing into 1 per cent. chloramine T after that solution had been applied four times within 60 minutes.

Experiments with Flavine. These yielded unsatisfactory results, and as the figures obtained are quite unreliable they will not be given.

It was necessary when investigating the antibacterial effect of such a slowly acting antiseptic to estimate the number of living bacteria after the fluid had remained in the wound for several hours. After such an interval a sputum-like membrane had formed on the walls of the wound, and during its formation most of the bacteria were found to have been caught up in its meshes, and in consequence it was quite impossible to emulsify them for the purposes of the test. The result of this was that the plate cultures made for the enumeration of the bacteria gave dense clumps of mixed colonies which it was impossible to count. Completely sterile cultures were never obtained; and the number of living microbes in the fluid appeared to increase the longer the sample of flavine solution remained in the wound, up to 15½ hours.

Conclusion. The above experiments show that, in the cases of Dakin's solution, eusol, and chloramine T, at any rate, no appreciable bactericidal effect was manifested upon the bacteria present in the wounds; in the case of flavine, the results were inconclusive, but showed, at any rate, nothing like a sterilization of the wound. This last finding was confirmed by bacteriological observations on many other infected wounds treated with this dye.

(4) Experiments carried out with a view to showing why the antiseptics in common use fail to sterilize infected wounds.

The fact that antiseptics fail, as has been shown above, to exert an appreciable bactericidal effect in the wound, suggests that their potency is in some way dissipated by contact with the walls of the wound, or its discharges, or by both these factors.

The following experiments were undertaken to show whether, in the case of Dakin's solution, eusol, chloramine T, and flavine, such deterioration of strength could be demonstrated when these solutions were placed in a wound, and, if so, to indicate the rapidity and degree of the change.

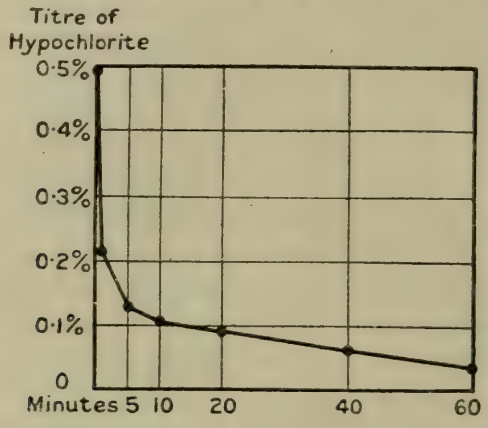
The destruction of antiseptic potency in wounds.

Experiment with Dakin's solution. The wound 'C' having been carefully washed out, it was filled with Dakin's solution and samples removed at intervals for titration of the hypochlorite against sodium thiosulphite and potassium iodide solutions as described in Carrell's book[1]; the technique being modified in order to make the method applicable to small volumes of fluid. The accuracy of using small volumes was tested by titrating repeatedly 10 c.c. and 0·1 c.c. volumes of Dakin's solutions, when it was found that the results corresponded closely. All estimations were carried out immediately the fluid was removed from the wound.

By reference to Fig. XIX it will be seen that after the Dakin's fluid had been in the wound for 5 minutes the concentration of hypochlorite had fallen to one-fourth of its original titre, and in 40 minutes it was only one-seventh of its original strength. When it is remembered that the wounds employed in carrying out these experiments

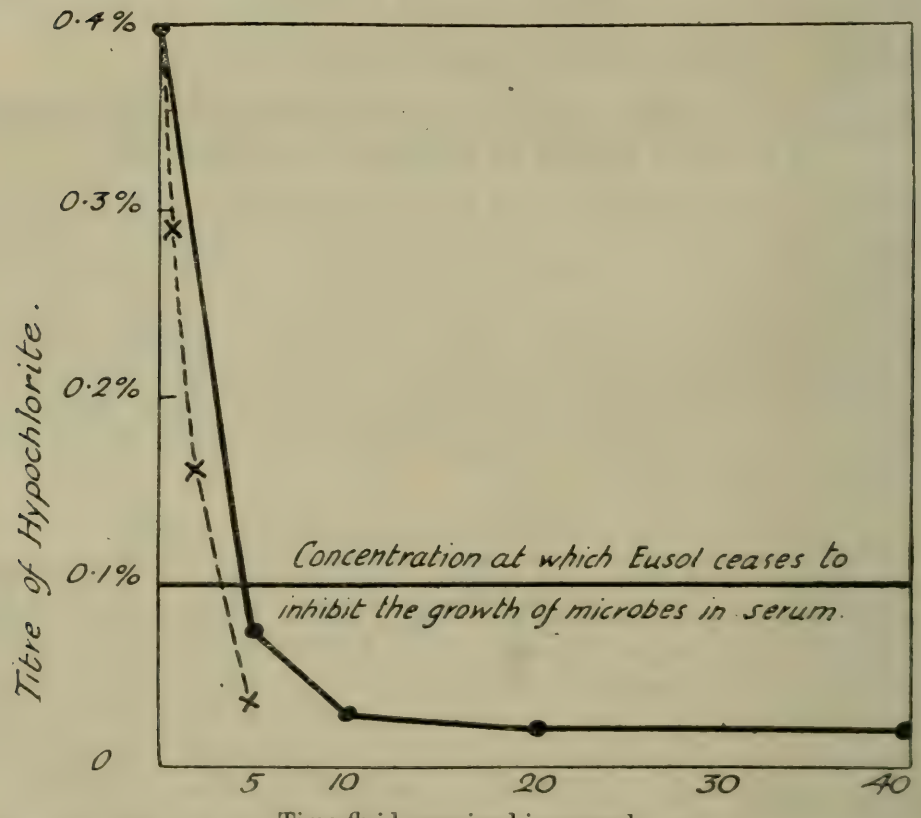
[1] Carrell et Dehelly, *Le traitement des plaies infectées*, Paris, 1917.

were cup-shaped depressions and in consequence the fluid in contact with the walls was only a small proportion of that contained in the wound, it will be seen that rapid as was this loss of strength of hypochlorite it would most probably have been even more rapid



Time fluid remained in wound.

FIG. XIX. Rate of destruction of Dakin's solution in a wound.



Time fluid remained in wound.

FIG. XX. Rate of destruction of eusol in a wound. Unbroken line shows result of Experiment 1. Broken line shows result of Experiment 2.

if the wound had been a comparatively flat surface, and the fluid had been syringed in under a dressing. This point will be raised again when dealing with eusol.

Experiments with Eusol. In these the hypochlorite was estimated by titration as above. (1) The result obtained in an experiment on wound 'C' is shown in Fig. XX.

This fig. shows the extraordinary rapidity with which the concentration of hypochlorites is diminished almost to a vanishing point. In 5 minutes the concentration of hypochlorites fell to about one-fifth, and in 10 minutes to one-tenth of the original strength. When these figures are compared with those giving the concentration of hypochlorite which inhibits the growth of micro-organisms (see p. 82) in a serous fluid, it will be seen that the antiseptic action of eusol applied to a wound is only momentary.

(2) Another experiment was carried out on a clean shallow flesh wound of about one week's standing. This wound had a capacity of about 2 c.c. of fluid.

Two c.c. of eusol were now introduced and kept constantly stirred, the technique in this way differing from that used in the first experiment when the eusol, after introduction, was left undisturbed. After an interval 1 c.c. of this fluid was withdrawn and immediately placed in 2 c.c. of a 10 per cent. potassium iodide solution. The cavity was now emptied and dried and 2 c.c. of fresh eusol introduced. After an interval 1 c.c. of this second application of eusol was withdrawn and immediately placed in potassium iodide solution. By such procedures specimens of eusol which had been in a perfectly clean granulating wound for $\frac{1}{2}$, 1, and 5 minutes respectively were obtained. On titrating these against thiosulphite of soda it was found that in 5 minutes the eusol had retained only one-tenth of its original concentration of hypochlorites. This result is shown by the broken line in Fig. XX.

Experiment with Chloramine T. The wound 'P' was washed and then filled with a 4 per cent. solution of the antiseptic. At intervals 0·2 c.c. samples were withdrawn for estimation of their strength by the addition of 1 c.c. of 4 per cent. acetic acid solution, which caused precipitation of the chloramine. By the amount of precipitate the destruction of chloramine could be roughly gauged as shown in the following table:

TABLE XXIII.

	Result of adding acetic acid.
Preliminary wash with chloramine T solution.	Heavy white precipitate.
Chloramine T solution after being in wound 5 min.	Ditto.
" " " " " 10 "	White floculent precipitate (much less).
" " " " " 20 "	No precipitate.
" " " " " 30 "	Ditto.
" " " " " 50 "	Ditto.

These results indicate that chloramine T is very rapidly destroyed when brought into contact with wound surfaces.

Experiment with Flavine. A 1 in 1,000 solution was introduced into the wound 'C' after it had been washed out, and was replaced by fresh solution after periods of contact with the wound varying from a few seconds up to 15½ hours.

The strength of flavine in each sample was estimated by matching the colour on filter paper of drops of definite volume against similar sized drops of solutions of known strength. The results are shown in Table XXIV.

TABLE XXIV.

Time during which flavine solution had remained in the wound.	*Concentration of flavine solution, a similar volume of which placed on filter paper gave the same colour.*
A few seconds	1 in 1,500
15 minutes	1 in 1,500
30 „	1 in 2,000
60 „	1 in 3,000
2 hours	1 in 3,500
4 „	1 in 3,500
15½ „	1 in 16,000

With regard to these results it must be remembered that the flavine solution was placed in a cup-shaped wound and in no way disturbed until the sample was withdrawn. Further, that before the last sample had been introduced the solution had already been applied to the wound (in several changes) for 7¾ hours, during which time the tissues had become heavily impregnated with the dye, and also coated with the sputum-like material which results from the action of the dye upon the discharges. These circumstances would tend markedly to delay the disappearance of the flavine from the solution, and this would have most probably been much more rapid if the solution had been applied in a thin layer to the wound walls, a condition that would obtain in wounds dressed in the usual manner.

Again, the conditions of the experiment differed from those of ordinary surgical practice in that no gauze or other dressings were applied to the wound. It will be shown later (p. 90) that cotton and other fabrics have the property of fixing very large quantities of flavine, so that when the solution is applied on gauze in the conventional manner of dressing a wound, the loss of strength of the dye must be very much greater and more rapid than was indicated in this experiment.

(5) Experiments in vitro bearing upon the bactericidal efficacy of antiseptics in the presence of serum.

The foregoing experiments have shown that Dakin's solution, eusol, chloramine T, and flavine quickly lose a large part of their bactericidal potency when introduced into a carefully cleansed and empty wound. Infected wounds do not remain long, however, in that condition. Exudation of serum is usually abundant, and the leucocytes emigrate—as will be shown—in great numbers, as soon as the strength of the antiseptic has been somewhat reduced. It therefore becomes necessary to determine what bactericidal effect any given antiseptic is capable of exerting in a medium consisting largely of serum, or serum mixed with leucocytes.

In the experiments which follow, the quenching effect of leucocytes, *per se*, upon antiseptic agents, has been left out of account, because it was impossible to obtain samples of pus which were in any way comparable. Serum alone has therefore been employed, or, rather—in order to approximate more closely to the conditions obtaining in infected wounds—serum to which has been added sufficient trypsin to just neutralize its anti-tryptic power.

Procedure and technique. It is, of course, necessary when testing antiseptics in serum to follow the routine of implanting the serum with microbes and then adding the antiseptic. This is especially important in connexion with the hypochlorites, which are very rapidly quenched by serum, since it would be manifestly unfair to such antiseptics to add them to the serum first and then at some later period to add the microbes. Trypsinized serum was therefore inoculated in bulk with the particular microbe chosen for the test and was then distributed in equal volumes into a series of small tubes, into each of which had previously been introduced the requisite quantity of progressively diluted antiseptic solution. Usually 9 volumes of serum were added to 1 volume of antiseptic; in a few cases equal quantities were employed. In all cases control tubes were put up, in which the antiseptic was replaced by water or normal salt solution, according as the antiseptic had been diluted in one or other of these fluids.

In a few experiments, where *Staphylococcus* was the organism used, the effect of the antiseptic was gauged simply by the complete prevention of growth in the serum, as judged by naked eye examination or subcultivations; more frequently gas-producing organisms were employed, and the amount of their growth in each of the mixtures was roughly indicated by measurement of the gas formed.

The technique employed for this latter purpose was practically that described by Beattie, in which melted vaseline is poured upon the top of the inoculated medium, thus making an air-tight plug which rises in the tube as the gaseous products of bacterial growth accumulate. Small test tubes made from glass tubing of a calibre of 4 to 6 millimetres were employed. For each experiment these tubes were made from the same length of selected glass tubing, so that the calibre of all the tubes used in any one experiment would be the same. Thus the measurement of the height of the column of gas in the different tubes was directly comparable and served as a comparative measure of the growth of the organism.

Although there are certain objections to this method of estimating the amount of growth of a microbe by the amount of gas formation, the method was shown to be sufficiently accurate by two series of tests.

1. The number of microbes were counted in tubes showing, respectively, little and much, gas formation; it was found that where there was more gas there were more microbes present.

2. On several occasions duplicate series of tubes were put up; after an interval of 24 hours the amount of gas produced was the same in each tube within very small limits.

In a few experiments conducted in this way, where the serum was inoculated with coliform bacilli (which do not make gas except in the presence of sugar) 1 per cent. of glucose was added to the trypsinized serum.

Results of the experiments. Two sets of data have been furnished by these experiments. In the first place figures were obtained giving, for each of the common antiseptics, the concentration which was necessary to inhibit all growth of the microbe tested. And secondly, data showing the reverse effect, viz. a *stimulation* of the growth of the microbe in serum by smaller concentrations of some of these antiseptics. These two effects may be considered separately.

Table XXV.

Antiseptic.	Micro-organism.											
	B. welchii.		*B. sporogenes.*		*B. tetanoides.*		*B. proteus.*		*B. coli.*		*Staphylococcus*	
	Concentration which completely inhibited growth.	*Highest concentration showing growth.*	*Concentration which completely inhibited growth.*	*Highest concentration showing growth.*	*Concentration which completely inhibited growth.*	*Highest concentration showing growth.*	*Concentration which completely inhibited growth.*	*Highest concentration showing growth.*	*Concentration which completely inhibited growth.*	*Highest concentration showing growth.*	*Concentration which completely inhibited growth.*	*Highest concentration showing growth.*
Iodine	1 in 500	1 in 1,000	1 in 500	1 in 1,000	1 in 500	1 in 1,000	1 in 500	1 in 1,000	1 in 500	1 in 1,000	1 in 500	1 in 1,000
Eusol	1 in 4	1 in 8	—	1 in 2	—	1 in 2	1 in 8	1 in 10	1 in 4	1 in 8	1 in 8	1 in 16
Dakin's solution	—	1 in 2	—	1 in 2	—	1 in 2	1 in 2	1 in 4	—	—	1 in 2	1 in 4
Chloramine T ..	1 in 60	1 in 120	1 in 50	1 in 100	1 in 80	1 in 160	1 in 50	1 in 100	—	—	—	—
Carbolic acid ..	1 in 200	1 in 400	1 in 200	1 in 400	1 in 200	1 in 400	1 in 200	1 in 400	1 in 200	1 in 400	1 in 200	1 in 400
Mercuric chloride	1 in 2,000	1 in 4,000	1 in 2,000	1 in 4,000	—	—	1 in 8,000	1 in 16,000	1 in 16,000	1 in 32,000	1 in 8,000	1 in 16,000
Malachite green	1 in 200	1 in 400	—	1 in 200	—	1 in 200	—	1 in 200	—	1 in 200	—	—
Flavine	1 in 16,000	1 in 32,000	—	—	—	—	1 in 1,000	1 in 2,000	—	1 in 1,000	1 in 16,000	1 in 32,000
											1 in 80,000	1 in 160,000
Brilliant green ..	—	1 in 1,000	—	—	—	—	—	—	—	1 in 1,000	—	—
Crystal violet ..	1 in 1,000	1 in 2,000	1 in 6,400	1 in 12,800	—	—	—	—	—	—	—	—

(*a*) Inhibitory Effect Exerted by Antiseptics on the Growth of Bacteria in a Serous Medium.

Table XXV sets forth the inhibitory power of some of the common antiseptics when allowed to act in a serous medium. If it is examined in relation to the experiments quoted in Sections (4), (6), and (9), which show that the effective strength of antiseptic solutions is largely dissipated by contact with the walls of the wound, the cellular exudates and the dressings, it will be realized that these solutions can seldom exercise in the wound, even for a few minutes, any destructive or inhibitory influence on the bacteria. In the case of Dakin's solution and eusol, for example, it has been shown on p. 78 that after 10 minutes in the wound these antiseptics have been reduced to at least one-tenth of their original strength, a concentration which is found incapable of even inhibiting the growth of microbes in serum. Similarly in the case of flavine, when allowance is made for fixation of the dye by the walls of the wound, the leucocytes and the dressings, it is clear that the ·1 per cent. solution ordinarily employed will be incapable of destroying or inhibiting *B. coli* or *B. proteus*, and it will not for long retain a strength of 1 in 16,000 which the table shows to be necessary in order to restrain the growth of the other bacteria.

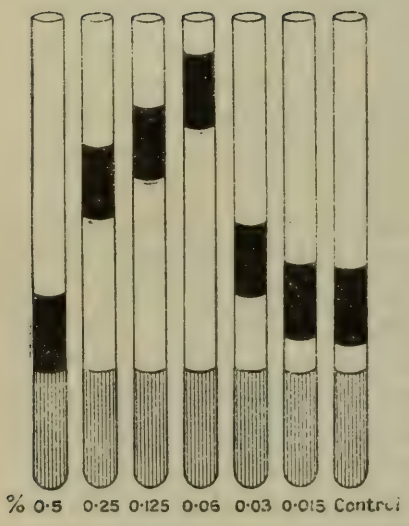
It will also be seen from this table that there is, with the commonly used antiseptics, very little variation in respect to the different organisms, whether they be spore-bearing or non-sporing bacteria. In connexion with the dye-stuffs it has long been known that members of the triphenyl-methane group, such as gentian violet, crystal violet, malachite green, &c., have a powerful bactericidal action in respect to the Gram-positive microbes, while they are very weak with regard to the Gram-negative organisms, such as *B. coli* or *B. proteus*. It was claimed for flavine that it had almost as powerful an action on *B. coli* as it had on the Gram-positive organisms, but this has not been confirmed by these experiments, for whereas it will in serum inhibit the growth of *Staphylococci* and *B. welchii* in a 1 in 16,000 dilution in serum, yet *B. coli* will grow and produce gas in a concentration of 1 in 1,000 and *B. proteus* in one of 1 in 2,000.

With the dye-stuffs it is impossible to give a definite figure for the inhibitory power, just as it is for their bactericidal power, as this varies so much with the number of microbes present. This difference of lethal concentration with the number of microbes acted on, manifests itself with all the common antiseptics, but whereas with a substance like carbolic acid it is very slight, with the dye-stuffs it is very marked. The extent of this difference is brought out in Table XXVI, which gives the results of adding different quantities of *Staphylococcus* to serial dilutions of carbolic acid, flavine, and crystal violet in nutrient broth. Two suspensions of microbes were used, one being 5 million times stronger than the other. Using carbolic acid the concentration necessary to inhibit growth with the stronger suspension was only twice that which inhibited growth with the weaker suspension, whereas with crystal violet it was sixteen times and with flavine at least sixty-four times as strong.

Table XXVI.

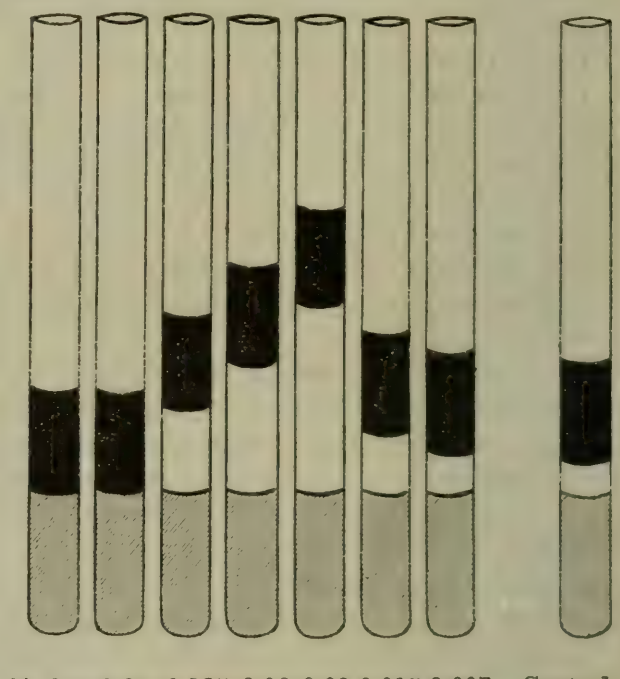
Antiseptic.	*Strong suspension of Staphylococci.*		*Weak suspension of Staphylococci.*	
	Least concentration which inhibited growth.	*Greatest concentration in which growth occurred.*	*Least concentration which inhibited growth.*	*Greatest concentration in which growth occurred.*
Carbolic acid	1 in 200	1 in 400	1 in 400	1 in 800
Crystal violet	1 in 160,000	1 in 320,000	1 in 2,500,000	1 in 5,000,000
Flavine	1 in 4,000	1 in 8,000	1 in 256,000	—

(*b*) The Stimulant Effect of Antiseptics on the Growth of Bacteria in Serum.



Concentration of carbolic acid.

Fig. XXI. Influence of carbolic acid on the growth of *B. welchii* in serum.



Concentration of carbolic acid.

Fig. XXII. Effect of carbolic acid on the growth of *B. sporogenes* in serum.

In Fig. XXI is seen a set of tubules in which the stimulant action exerted by weak carbolic acid solutions, e. g. 0·03 to 0·25 per cent. (as well as the inhibitory effect of a stronger solution), is well shown. The black columns represent the vaseline plugs. *B. welchii* was the organism employed. Fig. XXII illustrates a similar experiment in which *B. sporogenes* was used.

All the results which follow were obtained in a similar manner, but for simplicity of representation the gas formation has been plotted out in columns or curves. Further experiments with carbolic acid are shown in Figs. XXIII and XXIV.

Experiments with iodine solution. Iodine, as can be seen by reference to Fig. XXV, showed only an inhibitory action when tested with *B. sporogenes*. With *B. welchii* and *B. tetanoides*, however, there was evidence of stimulation in concentrations of 1 in 32,000 to 1 in 64,000, as is shown by Figs. XXVI and XXVII.

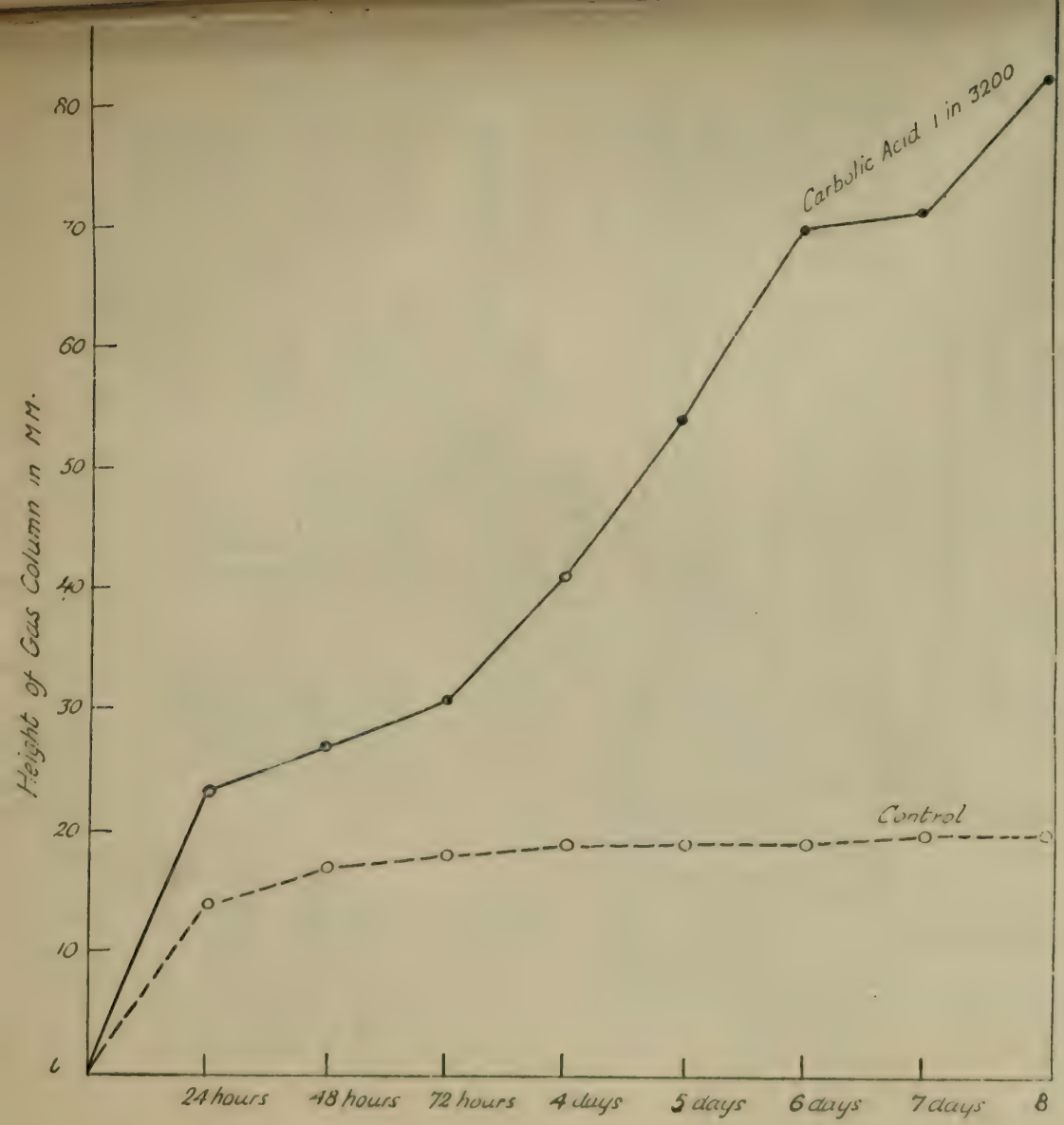
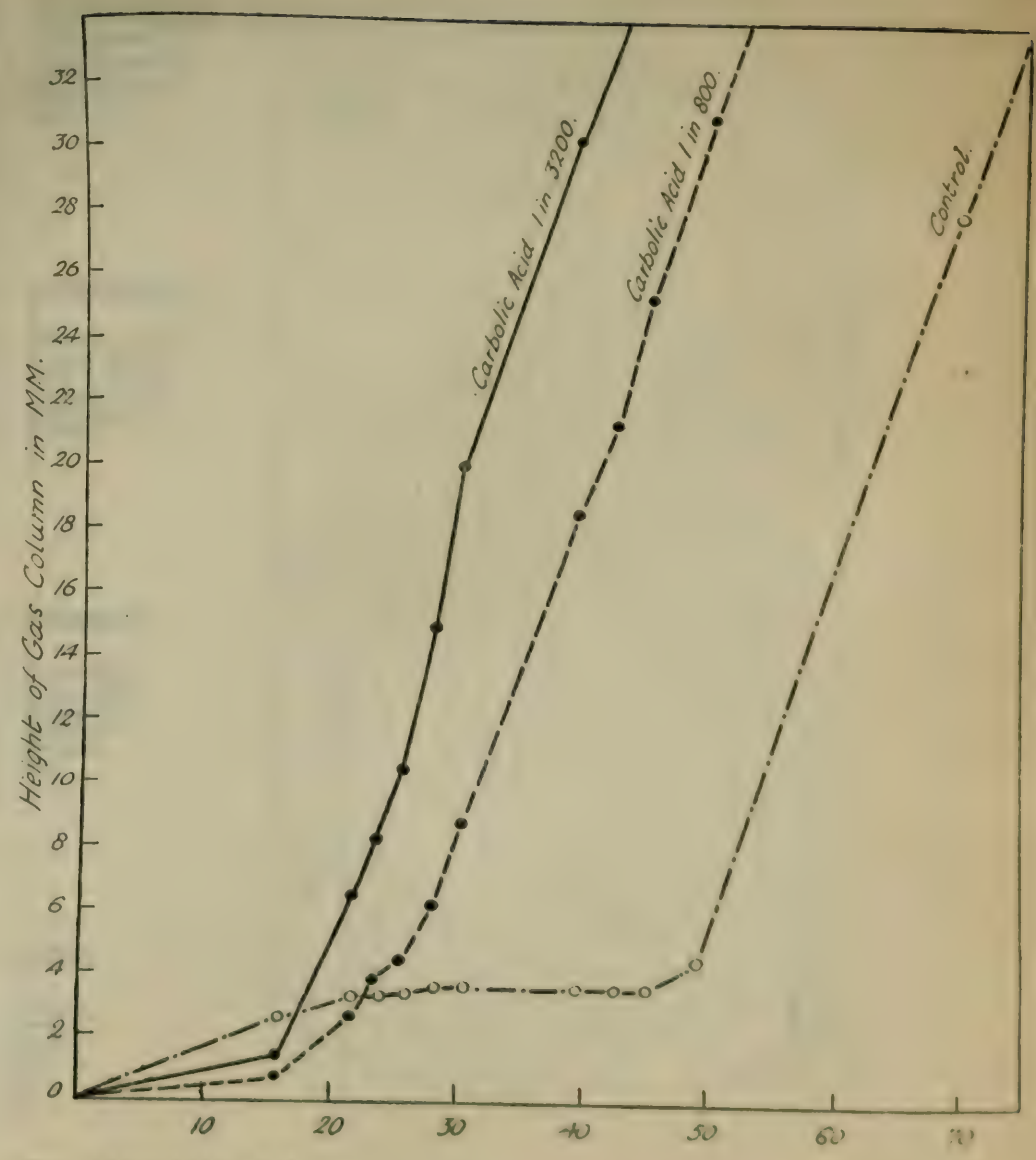


FIG. XXIII. Effect of carbolic acid on the growth of *B. welchii* in serum.



Time of incubation in hours.

FIG. XXIV. Effect of carbolic acid on the growth of *B. sporogenes* in serum.

Experiments with Eusol and Dakin's solutions. Eusol and Dakin's solution showed no stimulant action of *B. welchii*, whereas in the case of *B. sporogenes* the stimulant action was marked. Eusol also stimulated the growth of the *B. tetanoides*. These results are brought out graphically in Figs. XXVIII, XXIX, and XXX.

In the experiment illustrated by Fig. XXIX the implantation of microbes was small, so that the growth was very much delayed. In this case equal parts of serum and eusol proved a much better culture medium than did equal parts of physiological salt solution and serum. Growth started first in the tube containing equal parts of serum and eusol and, as can be seen by referring to the Fig., subsequently developed in the other tubes of the series, last of all in the control. In a case such as this, when a small number of microbes are implanted into unaltered serum the normal inhibitory action of the serum is sufficient to prevent growth until some change takes place, either due to the products of the microbes or to some other cause. It would appear that eusol markedly hastens this change.

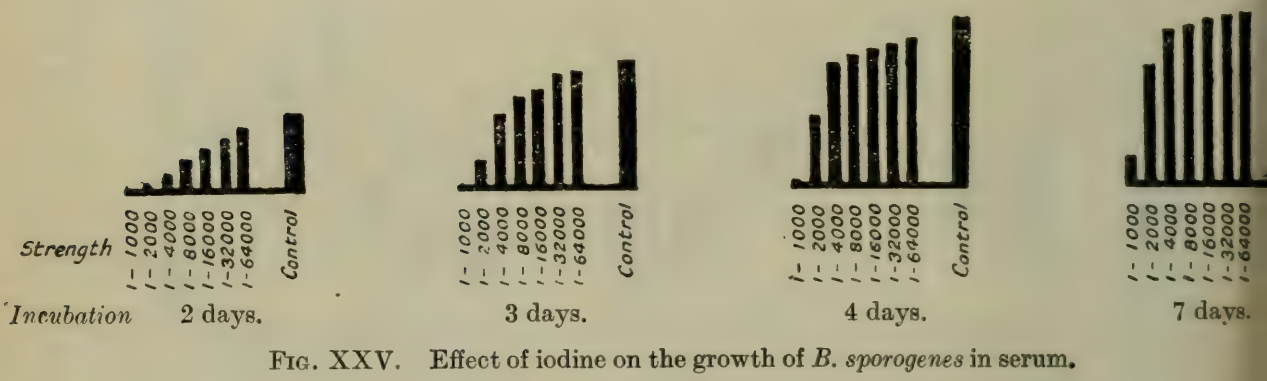


Fig. XXV. Effect of iodine on the growth of *B. sporogenes* in serum.

Experiments with Chloramine T. Chloramine T in concentrations of from 1 in 200 to 1 in 400 markedly stimulated the growth of all the spore-bearing anaerobic bacilli tested and, at the same time in rather weaker concentration, stimulated the growth of *B. proteus* (Figs. XXXI and XXXII).

Experiments with mercuric chloride and with flavine. No stimulant action on the growth of any of the bacteria tested was observed.

The information obtained from the above experiments is summarized in Table XXVII.

Table XXVII.

Stimulation of growth of bacteria by antiseptic solutions.

Antiseptic.	*Concentration of antiseptic which gave maximum stimulation of growth. Micro-organism used :*			
	B. welchii.	*B. sporogenes.*	*B. tetanoides.*	*B. proteus.*
Carbolic acid	1 in 3,200	1 in 800 to 1 in 6,400	1 in 6,400	None found
Mercuric Chloride ..	None found	None found	—	None found
Iodine	1 in 32,000	None found	1 in 64,000	—
Eusol	None found	1 in 2	1 in 16	—
Dakin's solution ..	None found	1 in 4	—	—
Chloramine T	1 in 240 to 1 in 480	1 in 320 to 1 in 640	—	1 in 640 to 1 in 2,560
Malachite green ..	1 in 12,800	None found	None found	1 in 400 to 1 in 6,400

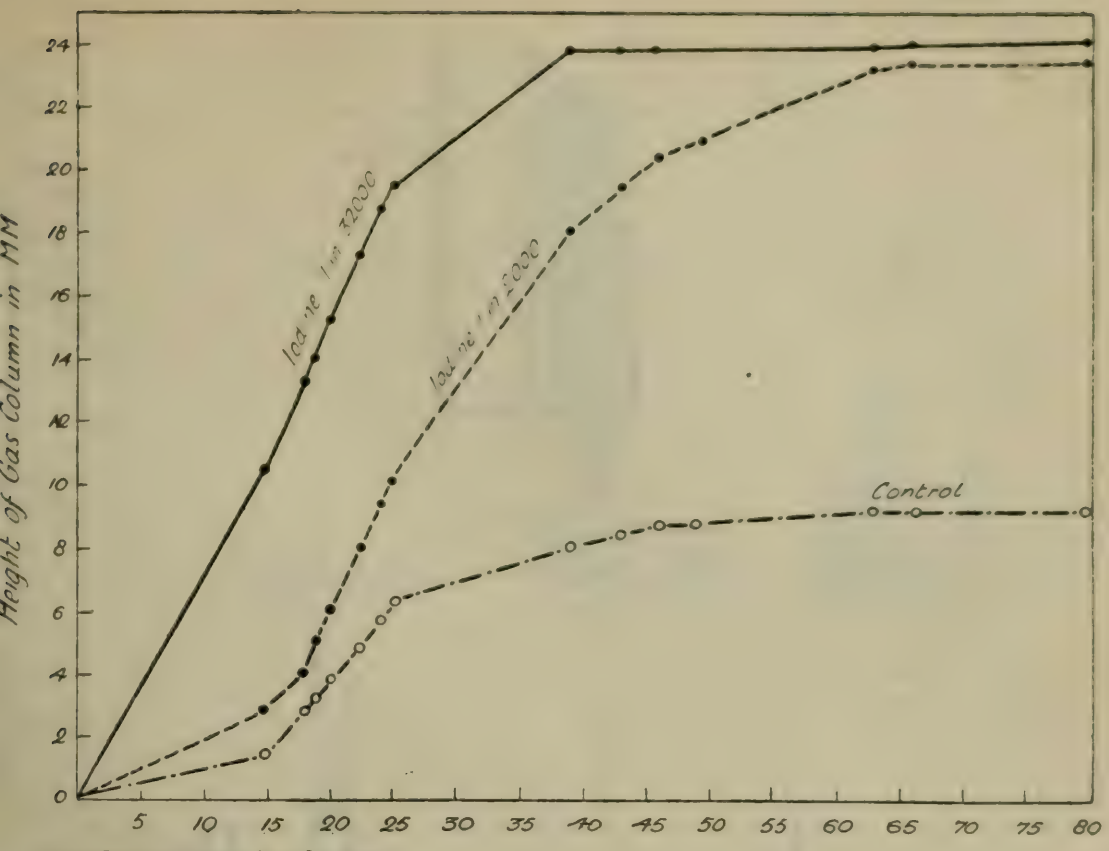


FIG. XXVI. Effect of iodine on the growth of *B. welchii* in serum.

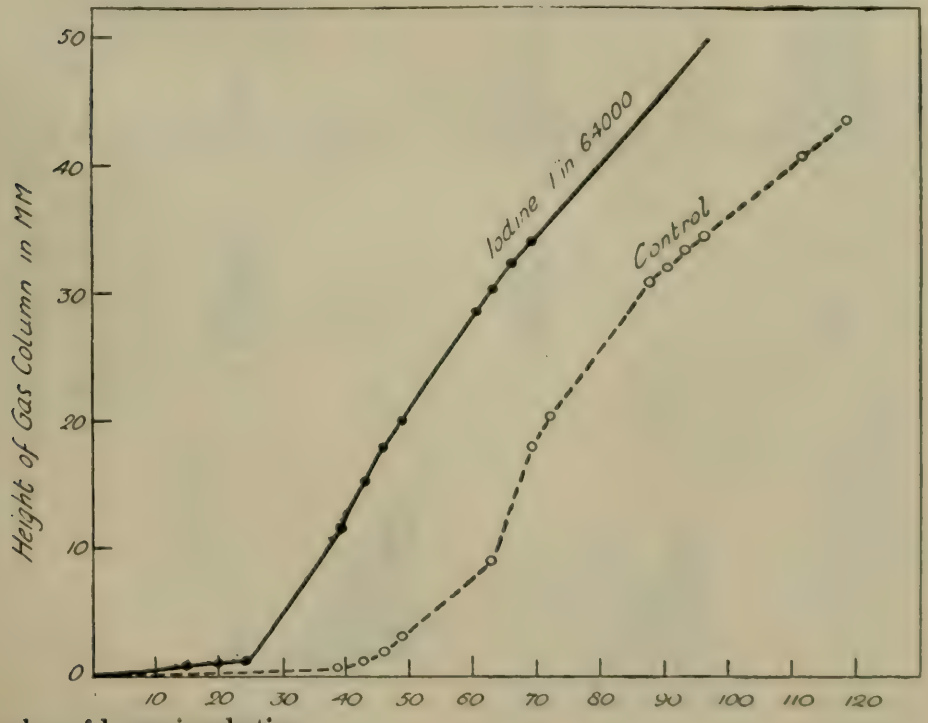


FIG. XXVII. Effect of iodine on the growth of *B. tetanoides* in serum.

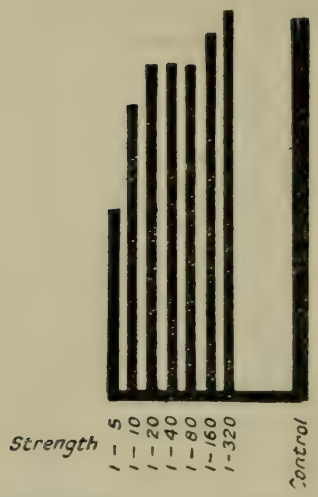


FIG. XXVIII. Effect of Dakin's solution on the growth of *B. welchii* in serum

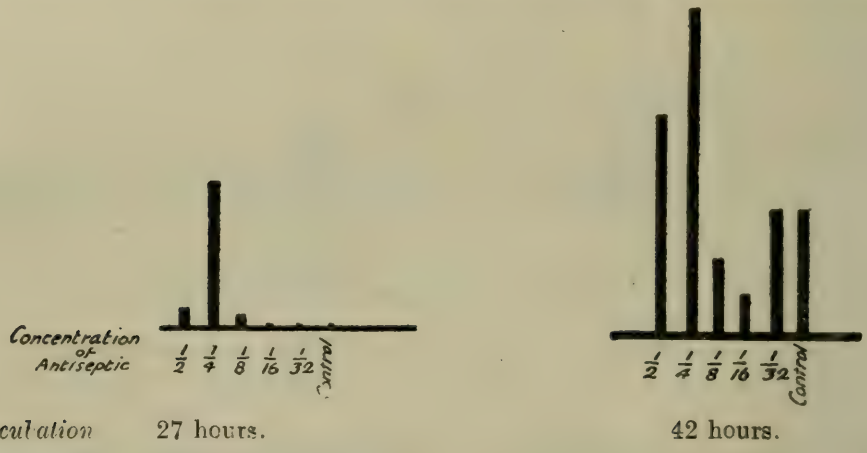


FIG XXVIII *a*. Effect of Dakin's solution on the growth of *B. sporogenes* in serum

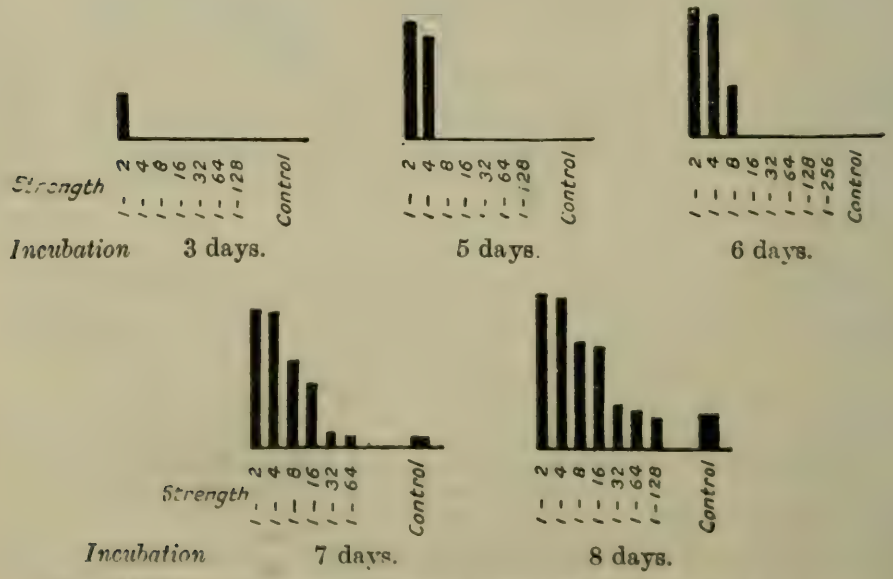


FIG. XXIX. Effect of eusol on the growth of *B. sporogenes* in serum.

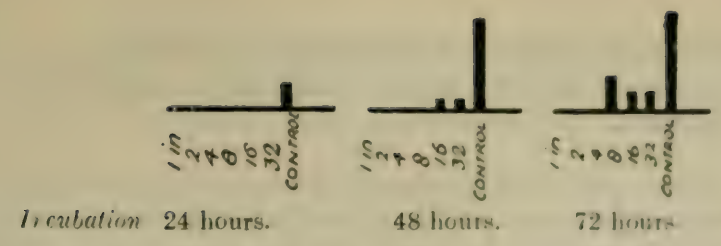


FIG. XXIX *a*. Effect of eusol on the growth of *B. welchii* in serum.

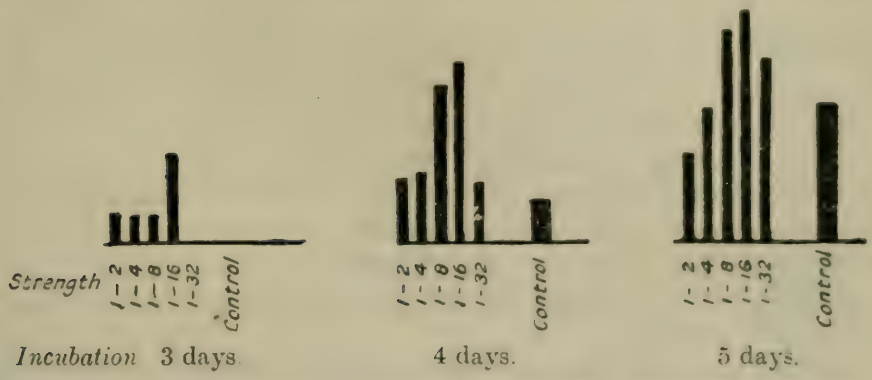


FIG. XXX. Effect of eusol on the growth of *B. tetanoides* in serum.

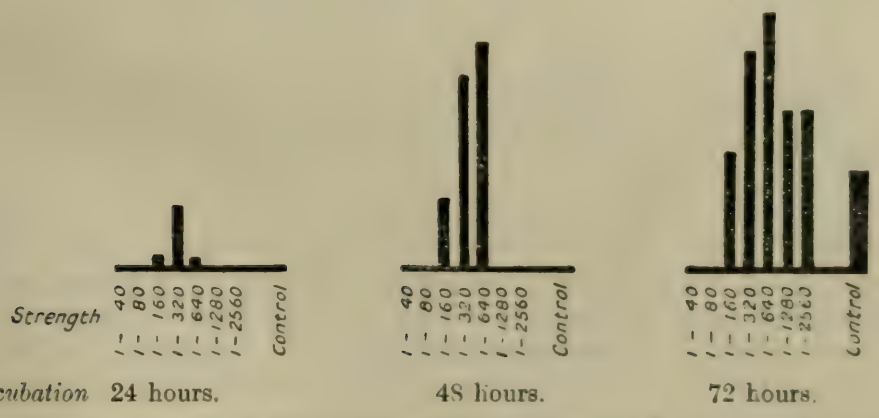


FIG. XXXI. Effect of chloramine T on the growth of *B. sporogenes* in serum

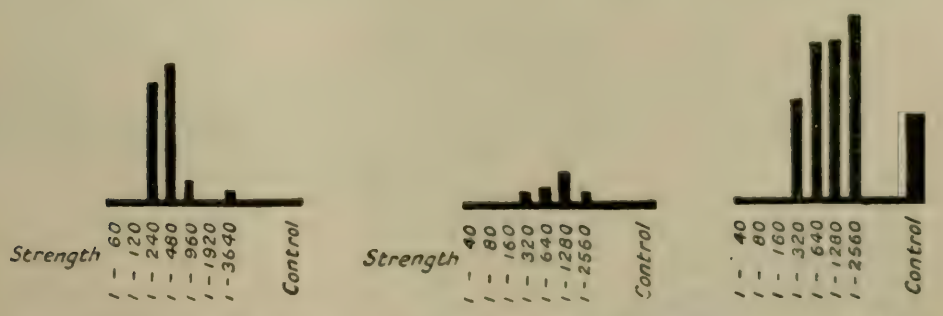


FIG. XXXII. Effect of chloramine T (*a*) on the growth of *B. welchii* in serum ; (*b*) on the growth of *B. proteus* in serum.

(6) Experiments which serve to demonstrate the fixation of certain antiseptic dye-stuffs by the fabrics commonly employed in the dressing of wounds.

In Sections (4) and (5) attention has been directed to the quenching of antiseptic solutions by contact with (*a*) the fixed tissues of the wound and (*b*) its serous discharges. It will now be shown that a further considerable loss of bactericidal potency is traceable—in the case of the antiseptic dyes—to the ready combination of the dye with whatever cotton or other fabric it encounters in the wound.

In the first series of experiments a small pad of gauze was allowed to soak up a few c.c. of the dye solution. After contact with the gauze for varying periods the solution was squeezed out of the pad and its dye-content ascertained by matching the colour of drops against that of similar drops of known solutions of the dye.

The following results were obtained :

(*a*) With brilliant green. 1 in 1,000.

After contact with the gauze for 1 minute the strength of the solution was 1 in 25,000.

After contact with the gauze for 3 hours the strength of the solution was 1 in 50,000.

(*b*) With flavine. 1 in 1,000. *Experiment* 1.

After contact for a few seconds the strength of the solution was 1 in 5,000.

After contact for 2 hours the strength of the solution was 1 in 10,000.

Experiment 2. With flavine solution. 1 in 4,000.

After contact for 5 minutes the strength of the solution was reduced to 1 in 128,000.

In a second series of experiments pieces of cotton bandage material of equal weight were packed into several lengths of glass tubing to form wads 2·25 cm. in depth. Equal quantities of various dye solutions were then poured into the tubes on one side of each wad and allowed to percolate through the bandage by gravity. Fixation of the dye by the cotton was indicated by the amount of fluid which passed through the wad uncoloured.

The results are shown in the following table :

TABLE XXVIII.

Antiseptic.	*Amount of filtrate passing through the bandage plug without any trace of colour.*
Flavine	1·7 cubic centimetres
Brilliant green	0·75 „ „
Crystal violet	1·3 „ „
Methylene blue	1·5 „ „
Neutral red	8 „ „
Acid Fuchsin (acid solution)	0
„ „ (alkaline solution)	0
Eosin	0 (First drop had a faint pink tinge.)
Phenol sulphonephthalein	0 (Tested by allowing to drop into alkali.)

(7) Experiments made to ascertain whether certain antiseptic solutions were capable of penetrating into—and killing the microbes contained within—small diverticula such as exist in all septic wounds.

The wounds of the war were all, in the early stage, irregular in shape and had many small pockets and processes passing off in different directions between the muscles or under the skin. In the late stages of superficial wounds the surface may be flat but the granulation tissue can be shown to contain many organisms below the surface.

An attempt was made in the experiments described below to reproduce these conditions, as far as possible, *in vitro*. Test tubes were converted into 'artificial wounds' by drawing out from their lower ends several spike-like processes about a centimetre in length. The resemblance to the actual wound condition was, of course, only rough; actually in the wound the processes are much longer and frequently they are more or less shut off from the general cavity by a narrow neck, whereas in the test tube they are widely open to the main cavity. This dissimilarity ought to have acted in favour of the antiseptic solution in our experiments.

By the aid of such 'artificial wounds' we were able to investigate the antiseptic action of fluids on bacteria in the cavity of a wound without the experiment being complicated by the presence of organisms in the tissues or by pieces of slough in the wound.



FIG. XXXIII. Test tube converted into an 'artficial wound'.

Experiment 1.

Two similar 'artificial wounds' were taken and into them was poured about 2 c.c. of human serum into which faeces had been planted. They were then incubated anaerobically for 24 hours. At the end of that time they contained a mixture of bacteria very similar to those seen in a recently inflicted wound, i. e. *B. welchii*, *Streptococci*, *Staphylococci*, coliform bacilli, and other micro-organisms.

One of the tubes was now 'dressed' with eusol, by the simple method of pouring off the contents, and replacing them with eusol. After repeating this performance several times, the eusol was allowed to stand in the tube for 20 minutes, when it was poured off and replaced with sterile human serum. The other tube was treated in exactly the same way, using physiological saline solution instead of eusol. The tubes were then replaced in the incubator under anaerobic conditions for 24 hours. This procedure was repeated every day for 12 days. Each day films were made from both tubes, and up to the end of 12 days there was practically no difference between the flora in the two tubes except that perhaps there was not such a copious growth of *B. welchii* in the tube which had been 'dressed' with salt solution.

Both tubes after 12 days showed *B. welchii, Streptococci, Staphylococci,* coliform bacilli, and many long thin Gram-negative bacilli.

This experiment would seem to show that eusol used as a single daily dressing is without *antiseptic* value in a wound, it is apparently incapable of penetrating into diverticula.

Experiment 2.

Tubes with processes similar to those used in Experiment 1 were filled in with serum planted with a faecal emulsion and incubated anaerobically for 24 hours. Films were made from each tube and examined microscopically and all showed the same flora. The tubes were now ' dressed ' with various fluids as under.

Tube	I.	' Dressed '	with	normal saline solution.
,,	II.	,,	,,	5 per cent. saline solution.
,,	III.	,,	,,	eusol.
,,	IV.	,,	,,	Dakin's solution.
,,	V.	,,	,,	carbolic acid, 1 in 40.

On the first day each of the tubes was washed with three changes of its corresponding fluid ; this was allowed to act for 20 minutes and was then poured off, replaced with serum, and the tubes incubated anaerobically for 24 hours. The results then were as follows :

			Bacteriological Flora.
Tube	I.	Much gas formation.	*B. welchii, B. coli, Streptococcus,* diphtheroids, *B. tetani.*
,,	II.	,, ,,	,, ,, ,, ,, ,,
,,	III.	,, ,,	,, ,, ,, ,, ,,
,,	IV.	,, ,,	,, ,, ,, ,, ,,
,,	V.	No gas formation.	Almost pure culture of *Streptococcus.* Few *B. coli.*

On the second day the ' dressing ' was carried out in a similar manner except that the fluids were allowed to act continuously for 1 hour after the preliminary washings. After 24 hours' incubation in serum the flora was unchanged. The next day the fluid was allowed to act for 3 hours, but the tubes after 24 hours' incubation showed the same flora. On the fourth day the fluid was allowed to act for 6 hours. After 24 hours' incubation the flora was the same, the Tube IV (Dakin's solution) showed the most copious growth. The next day the tubes were again washed with three changes of fluid, after which the fluids were allowed to act continuously for 3 days. The fluid was then poured off, replaced with serum and incubated for 24 hours. In Tubes I and II (normal and 5 per cent. salt solutions) all the microbes grew. Tubes III and IV grew apparently pure *Streptococcus.*[1] Tube V grew *Streptococcus* and Gram-negative bacilli. All these final cultures were diluted 3,000 times and 10 cmm. of the dilution was plated out and incubated aerobically. In all cases the amount of growth was approximately the same.

It was thought that possibly some of the bacteria, which had apparently been destroyed in Tubes III and IV, had not had time to grow out into the general mass of the culture fluid. All the tubes were, therefore, ' dressed ' again and the fluid was allowed to act

[1] Other organisms were also present as shown in the next stage of the experiment

for two days. The fluid was then poured off, replaced with equal parts of broth and serum, and the tubes incubated anaerobically for 2 days. The cultures were then examined with the following results:

Tube	I.	Gas formation.	*B. welchii*, *Streptococci*, coliform bacilli & *Staphylococci*
„	II.	„	„ „ „ „
„	III.[1]	„	„ „ „ „
„	IV.	„	„ „ „ „
„	V.	No gas formation.	*Streptococcus only.*

These experiments show that it is impossible to sterilize the cavity of a wound by washing it out with an antiseptic or by leaving that antiseptic in the wound for a long period. The reason of this is (apart from any question of bacteria in the tissues where the antiseptic does not reach them) that in the minute processes which are usually present in the recent wound the antiseptic does not penetrate, and in the intervals between the dressings the bacteria grow out again from these processes, so that in 24 hours the result is very much the same, whether an antiseptic has been used or not.

With the hypochlorite solutions (eusol and Dakin's solution) anaerobic and aerobic bacteria persisted after the tube had been left filled with the antiseptic for 3 days, and the fluid was then poured off and replaced by a fresh sample which remained in the tube for a further period of 2 days. The only difference between the tubes 'dressed' with these antiseptics and salt solution is that *Staphylococcus* persisted in the 'salt' tubes whereas they were not evident in the 'antiseptic' tubes. Of course in such an experiment it was not to be expected that there would be any change in the 'salt' tubes as the salt is not an antiseptic.

It is interesting to note that 1 in 40 carbolic at the very first 'dressing' seemed to destroy all the microbes except a few *B. coli* and *Streptococcus*. This latter organism, however, is the most dangerous one found in wounds, and it persisted in the 'carbolic' tube up to the end.

Experiment 3.

Tubes were next made so as to simulate a wound with infected walls in addition to the pockets. To imitate the tissues a small tightly-rolled ball of asbestos wool was employed; this was placed in a tube similar to those used in the first series of experiments.

The preliminary infection of this 'artificial wound' was made as before with faeces grown in serum for 24 hours; the 'dressing' was carried out in the same way as in the last experiments and the fluids were left in contact for the same times. The only difference was that no anaerobic precautions were taken.

Two tubes were used, one of which was 'dressed' with normal salt solution and the other with Dakin's solution. They were examined at the same times as in the previous experiment, and on every occasion there was much gas production and the flora remained as it was at the commencement of the experiment—even after 3 days' soaking in the antiseptic.

[1] Gas formation and the presence of *B. welchii* and *B. coli* were not evident in 24 hours, but were marked after 48 hours' incubation.

Experiment 4.

An imitation of an absolutely flat wound with infected tissue was next made. The tissues were represented by a ball of asbestos wool the size of a small pea and the wound by a small test tube without processes. Four such tubes were taken and the infection was made by incubating for 3 days aerobically in serum a mixed culture of *B. welchii, B. sporogenes, Streptococci,* and *B. coli.* In the presence of the ball of asbestos wool all these organisms flourished and there was much gas formation. The culture fluid was poured off and the tube and ball of asbestos wool was washed with four changes of the antiseptic fluid under investigation. The fluid was then left in the tubes for 3 days and the tubes were shaken at intervals. At the end of this interval the fluid was replaced by a mixture of serum and broth and the tubes were again incubated. The results were as follows :

TABLE XXIX.

Dressing fluid used.	
Normal salt.	Copious growth & gas formation. All the microbes have grown.
Eusol.	„ „ „ „ „
Dakin's solution.	„ „ „ „ „
Alcohol 90 per cent.	No growth in 24 hours, but in 3 days' growth of anaerobes and *Streptococci.*

One would have supposed that a small ball of asbestos wool would have been less difficult to penetrate than the infected tissues round a wound, but here it is shown that none of the antiseptic fluids used were capable of sterilizing even a small asbestos ball saturated with a serous fluid.

The following experiments were made to ascertain if certain dyes would show better results than the antiseptic used in the above experiments.

Experiment 5.

' Artificial wounds ' made out of small test tubes were filled with serum implanted with faeces.

After being grown anaerobically for 24 hours, there was much gas formation and films showed the ordinary faecal flora : *Streptococci,* diplococci, coliforms, large and small Gram-positive organisms.

The tubes were then emptied, filled in with antiseptic and kept for 24 hours at 37° C. The antiseptic was removed and the tubes, having been washed out with normal salt solution several times, were filled with glucose broth and replaced in the incubator for 24 hours. The results were as follows :

Tube I. Flavine 1 per cent. solution was added to make 1 in 1,000 in the original serum culture of faeces.

Result after final incubation. Growth with gas formation ; film showed *B. coli, Streptococci,* and Gram-positive bacilli.

Tube II. Faecal culture emptied out. Tube washed with flavine 1 in 1,000 several times, then filled with 1 in 1,000 in water.

Result after final incubation. Growth with gas formation ; film showed *B. coli.*

Tube III. Tube emptied of faecal culture, washed with flavine 1 in 1,000 several times, then filled with a gauze plug soaked in flavine 1 in 1,000 in serum.

Result after final incubation. Growth with gas formation ; film showed *B. coli*, *Streptococci*, and Gram-positive bacilli.

Tube IV. Brilliant green added to faecal culture to make 1 in 1,000.

Result after final incubation. Growth with gas formation ; film showed *B. coli*, *Streptococci*, and Gram-positive bacilli.

Tube V. Faecal culture emptied out. Tube washed with brilliant green 1 in 1,000 in water several times, then filled with brilliant green 1 in 1,000 in water.

Result after final incubation. Growth with gas formation ; film showed *B. coli*, *Streptococci*, and Gram-positive bacilli.

Summary.

1. In a test tube with small processes, which simulate the pockets present in a wound cavity, a daily ' dressing ' with eusol has no effect on the bacterial flora.

2. In such ' artificial wounds ' eusol and Dakin's solution are incapable of sterilizing the tubes even after acting continually for three days.

3. Carbolic acid, 1 in 40, is incapable of killing the *Streptococci* in such ' artificial wounds ' even after acting continually for three days.

4. Antiseptic dye-stuffs, such as flavine, 0·1 per cent., and brilliant green, 0·1 per cent., acting for 24 hours, similarly failed to sterilize such ' artificial wounds '.

5. Eusol, Dakin's solution, and alcohol (90 per cent.) are incapable of penetrating a small ball of asbestos wool impregnated with a serous fluid.

6. In view of these experiments it is unreasonable to expect Dakin's solution, eusol, carbolic acid, flavine, or brilliant green to sterilize the cavity of a wound if there are any small processes present, or if the tissues around are infected.

(8) Experiments dealing with the ability of certain antiseptics to penetrate the walls of the wound.

No experiments bearing directly upon this problem have been carried out. In the first days of the war, however, when considerable discussion was taking place on the possibility of obtaining a rapid sterilization of wounds by the early application of a strong antiseptic, a series of observations was made upon the ability of antiseptics to penetrate into blood clot. Inasmuch as this process may be held to resemble, in some measure, the process of penetration into organized tissues, our findings may be here recorded.

In planning the experiments we proceeded upon the reflection that a chemical sterilization, such as was contemplated, could only be achieved if the antiseptic employed was able to kill bacteria and spores embedded in blood clot, for in every wound there occurs a certain amount of oozing of blood, if not definite haemorrhage, and most of the microbes are presumably caught up in the resulting blood clot. It was, therefore, necessary to test the feasibility of

killing bacteria and spores embedded in blood clot. The antiseptic to be tested was used in a proportion (arbitrarily chosen) of one to four of blood (clot in serum)—a proportion which, it was thought, might fairly well represent the conditions obtaining in a wound when the latter had been ' opened up ' under an anaesthetic.

Technique. Into a number of small test tubes of approximately similar bore (·8 cm.) were introduced (1) a 10 cmm. volume of a broth culture of *Staphylococcus, Streptococcus* or other organism, or a dilution of such a culture ; (2) one cubic centimetre of blood freshly drawn from a human vein.

The tubes, after the contents had been thoroughly mixed, were then put aside, usually at 37° C., for an hour or two, to allow a firm clot to form, exude its serum, and shrink away from the sides of the tube. Such clots were of the shape and size shown in Fig. XXXIV and usually were about $\frac{1}{2}$ to $\frac{3}{4}$ cubic centimetre in volume.



Fig. XXXIV.

To each of the tubes with its one cubic centimetre of serum and clot was then added 0·25 cubic centimetre of the antiseptic to be tested, which was duly mixed with the serum by shaking. In some cases this mixing was far from perfect owing to the very dense viscid precipitate immediately thrown down.

After exposure of the clots to the antiseptic in serum for a period of several hours at 37° C., each was in turn picked out and washed in several changes of sterile salt solution with a view to diluting any antiseptic carried over. Each clot was then broken up with a stout platinum wire bent at an angle and several fragments were planted into tubes of sterile nutrient broth in order to ascertain if the organisms in the clot had all been killed.

The approximate number of organisms originally present in each clot was usually ascertained by making appropriate dilutions of the broth culture employed and plating sample volumes of these.

Table XXX.

Phenol :	*Staphylococcus.*	*Streptococcus.*	*B. sporogenes.*	*B. tetani.*	*Approximate number of organisms in the clot.*
90 per cent. B.P.	Killed.	..	Not killed.	..	*Staphylococcus* 55,000 : *B. sporogenes* 6,000.
90 ,, ,,	..	Killed.	Killed (15 hrs. exposure to antiseptics).	..	*Streptococcus* 12,000 : *B. sporogenes* 10,000.
90 ,, ,,	..	..	Not killed.	..	*B. sporogenes* 1,000.
90 ,, ,,	..	..	Not killed.	..	*B. sporogenes* 1,000.
50 per cent. with glycerine and almond oil.	..	..	Not killed.	..	*B. sporogenes* 1,000.
30 per cent. with almond oil and liquid paraffin.	..	..	Not killed.	..	*B. sporogenes* 50,000.
20 per cent. with almond oil.	..	Killed.	..	Not killed.	*B. tetani* 50,000 : *Streptococcus* not determined.

Table XXX (*continued*).

Phenol:	*Staphylococcus.*	*Streptococcus.*	*B. sporogenes.*	*B. tetani.*	*Approximate number of organisms in the clot.*
20 per cent. suspended with Tragacanth.	..	Killed.	..	Not killed.	*B. tetani* 50,000; *Streptococcus* not determined.
10 per cent. in spirit	..	Killed.	..	..	*Streptococcus* not determined.
10 per cent. in spirit.	..	Killed.	..	..	*Streptococcus* 3,000,000.
10 per cent. with formalin 5 per cent., with alcohol and glycerine.	Not killed.	..	..	..	*Staphylococcus* 166,000.
10 per cent. with formalin 5 per cent.	..	Killed.	Not killed.	..	Not determined.
5 per cent. aqueous.	Inhibited.	..	..	..	*Staphylococcus* 50,000.
5 per cent. aqueous.	..	Not killed.	..	..	*Streptococcus* 3,000.
2·5 per cent.	..	Not killed.	..	..	*Streptococcus* 1,000.
Lysol:					
10 per cent. with Tragacanth suspension.	Not killed.	..	Not killed.	..	*Staphylococcus* 55,000; *B. sporogenes* 6,000.
Cresol:					
30 per cent. with almond oil and liquid paraffin.	..	..	Not killed.	..	*B. sporogenes* 50,000.
20 per cent. with almond oil and liquid paraffin.	..	Killed.	..	Not killed.	*Streptococcus* not determined; *B. tetani* 50,000.
20 per cent. with Tragacanth.	..	Killed.	..	Not killed.	*Streptococcus* not determined; *B. tetani* 50,000.
10 per cent. with Tragacanth.	Killed.	..	Not killed.	..	*Staphylococcus* 55,000; *B. sporogenes* 6,000.
10 per cent. with liquid paraffin.	Killed.	..	Not killed.	..	*Staphylococcus* 55,000; *B. sporogenes* 6,000.
10 per cent. with liquid paraffin.	..	Not killed.	Not killed.	..	*Streptococcus* 12,000; *B. sporogenes* 10,000.
10 per cent. with liquid paraffin and almond oil.	..	Inhibited.	..	..	
10 per cent. with spirit.	..	Killed.	..	..	*Streptococcus* 3,000,000.
5 per cent.	..	Not killed.	Not killed.	..	
Mercury Salts:					
Perchloride, 1 in 1,000 aq.	..	..	Not killed.	..	*B. sporogenes* 1,000.
,, 1 in 1,000 aq.	Not killed.	..	..	..	*Staphylococcus* 50,000.
,, 1 in 500 aq.	Killed.	Killed.	..	..	*Staphylococcus* 48,000; *Streptococcus* 180,000.
,, 1 in 500 aq.	..	Not killed.	..	..	*Streptococcus* 15,000.
Biniodide, 1 in 500 aq.	Not killed.	..	..	..	*Staphylococcus* 166,000.
,, 1 in 500 aq.	..	Not killed.	..	..	*Streptococcus* 15,000.
,, 1 in 500 aq. with spirit in equal parts.	..	Killed.	..	..	*Streptococcus* 3,000,000
Iodine:					
Tincture iodine, undiluted.	Not killed.	..	Not killed.	..	*Staphylococcus* 55,000; *B. sporogenes* 6,000.
,, ,, ,,	..	Not killed.	Not killed.	..	*Streptococcus* 12,000; *B. sporogenes* 10,000.
,, ,, ,,	..	Killed.	..	..	*Streptococcus* 3,000,000.
,, ,, ,,	..	Killed.[1]	..	..	*Streptococcus* 15,000.
,, ,, ,,	..	Killed.[1]	..	..	
Formaldehyde:					
0 per cent. (50 per cent. of formalin).	..	Killed.	..	Killed.	*Streptococcus* not determined; *B. tetani* 50,000.

[1] When a ten-fold stronger emulsion of *Streptococcus* was added to the blood the result was 'not killed'.

TABLE XXX (*continued*).

FORMALDEHYDE:	*Staphylococcus.*	*Streptococcus.*	*B. sporogenes.*	*B. tetani.*	*Approximate number of organisms in the clot.*
20 per cent. (50 per cent. of formalin).	..	Killed.	Not killed.	..	*Streptococcus* 40,000; *B. sporogenes* 100,000
15 per cent.	..	Killed.	Not killed.	..	*Streptococcus* 40,000; *B. sporogenes* 700,000
12·5 ,,	..	Killed.	Not killed.	..	*Streptococcus* 40,000; *B. sporogenes* 100,000.
10 ,,	..	Killed.	Not killed.	..	*Streptococcus* 40,000; *B. sporogenes* 100,000.
10 ,,	Killed.	Killed.	..	..	*Staphylococcus* 48,000; *Streptococcus* 180,000.
10 ,,	..	..	Not killed.	..	*B. sporogenes* 50,000.
10 ,,	..	..	Not killed.	..	*B. sporogenes* 1,000.
5 ,,	..	..	Not killed.	..	
5 ,,	Not killed.	..	Not killed.	..	*Staphylococcus* 220,000; *B. sporogenes* 200,000.
5 ,,	..	Killed.	Not killed.	..	*Streptococcus* 40,000; *B. sporogenes* 100,000.
5 ,,	Inhibited.	..	..	..	
PERMANGANATE OF POTASH:					
5 per cent. aqueous.	..	Not killed.	..	..	*Streptococcus* 3,000.
5 ,, ,,	..	..	Not killed.	..	*B. sporogenes* 1,000.
1 ,, ,,	..	..	Not killed.	..	*B. sporogenes* 1,000.
METHYLATED SPIRIT:					
Undiluted.	..	Inhibited.	..	..	
,,	..	Not killed.[1]	..	..	Not determined.
,,	..	Killed.	..	..	*Streptococcus* 15,000
,,	..	Killed.	..	..	*Streptococcus* 1,000,000.

(9) Investigation of certain changes brought about in the tissue and blood cells as the result of the introduction of antiseptics into the wound.

It was laid down in Section 1 that the ideal antiseptic 'must not exercise any local destructive action on the tissues, or interfere with the physiological properties of the tissue fluids or cells'.

(*a*) THE ACTION OF ANTISEPTICS UPON THE FIXED TISSUES.

The only observations which appear worthy of record in this connexion are the following:

(*a*) Solutions of malachite green and of brilliant green were not found to be inimical to the growth of epithelium in the wounds, but rather stimulated it (vide clinical section, Case No. 2). The strength in which these solutions actually came into operation upon the tissues cannot be stated, as a large part of their strength (0·1 per cent. solutions were employed) must have been expended upon combination with the cotton dressings (vide section 6).

(*b*) The only manifest destruction of fixed tissues was seen in the case of flavine. A 0·1 per cent. solution of this dye was applied on muslin to a superficial wound upon which epithelium was growing rapidly around several recent skin grafts. After 2 days nearly all the new-formed epithelium had been destroyed.

[1] With 1,000-fold weaker planting, result was 'killed'

In connexion with another wound—the cup-shaped wound 'C'—it was observed that the normal rate of exudation from the walls was reduced by 50 per cent. after the tissues had been exposed to flavine for some hours.

(*b*) The Destructive Effect of Antiseptics on Leucocytes.

The leucocidal action of antiseptics has been tested in several ways: (1) by introducing a volume of the antiseptic solution as a fourth volume in an 'opsonic mixture', incubating for 15 or 20 minutes and then estimating the amount of phagocytosis; (2) by estimating the power of the leucocytes to emigrate in the presence of the antiseptic; (3) by mixing blood with the antiseptic and then testing, at intervals, the capacity of the leucocytes to ingest microbes.

The first method has been frequently employed, but it is of little use as a guide to the toxicity of an antiseptic for practical purposes. Antiseptics vary very much in the rapidity of their action on leucocytes, just as they vary in the speed with which they kill bacteria. If 15 or 20 minutes is allowed for the action of the antiseptic on the leucocytes, only those fluids which have a very rapid action on the white corpuscles (and probably also on bacteria) will show their full effect. Again it must be remembered that in such an experiment phagocytosis is going on all the time, so that the resultant number of ingested bacteria is in no way an indication of the capacity of the leucocytes to ingest bacteria *after 20 minutes' contact with the antiseptic*. The greater portion of the phagocytosis occurs during the first 5 or 10 minutes of incubation. By using this test an antiseptic which has a very slow toxic action on the leucocytes, such as flavine, compares very favourably with quick-acting antiseptics such as the hypochlorites.

The same objection applies, to a less extent, to the method of measuring the amount of emigration in Wright's emigration tubes.[1] In this method emigration commences rapidly and reaches its maximum (where there is no special attractive force) in something like two hours. Thus the very slow acting antiseptics, such as flavine, do not show, even by this method, their full effect. (When the tubes are examined under the microscope, however, after, say, three hours' incubation, the emigrated leucocytes in tubes containing flavine show up as rounded yellow bodies in sharp contrast to the actively moving forms in the control tubes.) It will be seen, however, from Table XXXI that the antiseptics which act with moderate rapidity, such as carbolic acid and perchloride of mercury, show by this method practically their full toxic effect on the leucocytes.

The best method of examining the effect of antiseptics on leucocytes is undoubtedly to mix the antiseptic dilution with defibrinated blood and allow this mixture to stand in the incubator at 37° C. At intervals samples are removed and the physiological state of the leucocytes is arrived at by comparing their capacity to ingest microbes with the phagocytic capacity of leucocytes from blood treated in exactly the same way with physiological salt solution

[1] *Lancet*, 1915, i. 737, 787, 843, 897.

substituted for antiseptic. Two volumes of the blood-antiseptic mixture is mixed with one volume of previously opsonized microbes and incubated for 10 or 15 minutes, after which the amount of phagocytosis which has taken place is estimated. The same is done with the control blood without antiseptic. By testing in this way at long and short intervals, a true picture of the action of any antiseptic on leucocytes is obtained.

From Table XXXI it appears that when this method is employed to test the slow-acting antiseptics, such as flavine and brilliant green, and 24 hours' contact is allowed, the latter dye is only one-tenth as toxic to leucocytes as flavine; when, on the other hand, the same solutions are tested by 2 hours' contact in Wright's emigration tubes,[1] brilliant green is found to be 4 times as toxic as flavine. It may be noted that Browning[2] also, using the opsonic method and 20 minutes' contact of the antiseptic with the leucocytes, found brilliant green to be 4 times as toxic as flavine.

The table also shows that whereas carbolic acid and flavine manifested the same toxicity to leucocytes when acting for only 20 minutes, flavine was found to be 1,000 times as toxic as carbolic acid when the antiseptic was allowed to act on the cells for 24 hours.

Table XXXI.

Showing the effect of Antiseptics on Leucocytes.

Antiseptic.	Method I.[3]	Method II.	Method III.		
	Time of contact of antiseptic and leucocytes 20 minutes. Concentration which reduced the amount of phagocytosis by half.	*Time of contact of antiseptic and leucocytes 2 hours. Greatest concentration which permitted emigration of leucocytes.*	*Concentration which reduced the phagocytic activity of the leucocytes by half. Time of contact of antiseptics and leucocytes.*		
			2 hours.	*5 hours.*	*24 hours.*
Carbolic acid ..	1 in 500	1 in 1,600	1 in 1,600	1 in 1,600	1 in 2,000
Mercuric chloride..	1 in 7,000	1 in 16,000	1 in 16,000	1 in 16,000	1 in 20,000
Iodine	1 in 3,500	1 in 200	1 in 500	1 in 1,000	1 in 1,000
Eusol (available Cl)	1 in 4,000	1 in 2,400	—	—	—
Dakin's solution (available Cl) ..	1 in 4,000	1 in 3,600	1 in 3,600	1 in 3,600	1 in 3,600
Flavine	1 in 500	1 in 4,000	1 in 1,000	1 in 500,000	1 in 2,000,000
Brilliant green ..	1 in 2,000	1 in 16,000	1 in 5,000	1 in 20,000	1 in 160,000
Malachite green ..	1 in 7,000	1 in 16,000	1 in 6,000	1 in 12,000	1 in over 100,000
Crystal violet ..	1 in 7,000	1 in 16,000	—	—	—

It is to be noted also that the migratory power of leucocytes provides a more delicate test than does their phagocytic activity. This is shown especially in connexion with the aniline dyes by a comparison of the second and third columns, where, in both cases, 2 hours was the time of contact of the antiseptic and the leucocytes. With each of the dyes examined the greatest concentration in which

[1] *Lancet*, 1915, i. 737, 787, 843, 897.

[2] Browning, Gullbransen, Kennaway, and Thornton, *Brit. M. J.*, 1917, i. 73.

[3] These figures are taken from an article by Browning, Gullbransen, Kennaway, and Thornton (*Brit. Med. J.*, 1917, i. 73).

emigration occurred was from 3 to 4 times weaker than the concentration which reduced the amount of phagocytosis by half.

This would seem to show that in 2 hours certain concentrations of these slow-acting substances damage the leucocytes to such an extent that they prevent active emigration, although they are still sufficiently alive to ingest microbes suitably offered to them. It must be remembered that phagocytosis is only a stage in the destruction of the microbe, and it is probable that these damaged leucocytes would be incapable of completing the work of destruction which is only commenced by the ingestion of the bacteria.

(10) The effect produced by certain antiseptics upon the exudation of fluid into the wound—and upon the properties of that exudate.

In spite of the failure to achieve the sterilization of any infected wound by a single application of an antiseptic—or indeed to show that antiseptics exert any appreciable bactericidal effect when repeatedly applied—it has been widely maintained that the employment of certain of them in the treatment of infected wounds has been beneficial. The question therefore arises—are these substances capable of exerting some beneficent effect distinct from a directly anti-bacterial action ?

One of us (A. F.)[1] has suggested that, by virtue of a mild irritant action on the tissues, certain antiseptics provoke an increased flow of the normal exudate into the wound, and that to this exudate—of fluid and leucocytes—should be ascribed any beneficial effect that is forthcoming. To such an indirect action of antiseptics Fleming has applied the term ' physiological '. With this conception in view the following experiments were carried out, in order to determine :

(*a*) The rate of exudation into the wound following the application of various antiseptics.

(*b*) The albuminous content of such exudates.

(*c*) The leucocyte content of such exudates.

(*d*) The anti-tryptic value of such exudates.

(*a*) The Rate of Exudation.

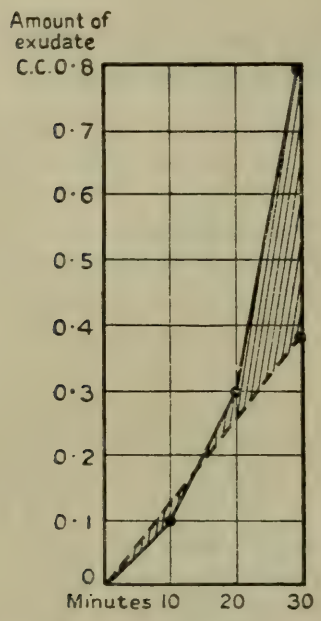
Experiments with chloramine T solution. (1) The normal exudation into the cup-shaped wound ' P ' having been first determined, as described on p. 74, a 4 per cent. solution of chloramine T was applied for varying periods, at the end of each of which the increase of fluid in the cavity was carefully estimated. The result is graphically shown in Fig. XXXV.

This chart shows that the rate of flow of the exudate for the first 5 and 10 minutes is practically the same as that into the empty cavity ; the amount exuded in 30 minutes shows a very marked increase. This is most probably due to the irritant action of the antiseptic solution, and such an assumption is borne out by the nature of the

[1] Fleming, *Brit. J. Surg.*, 1919, 7, 99.

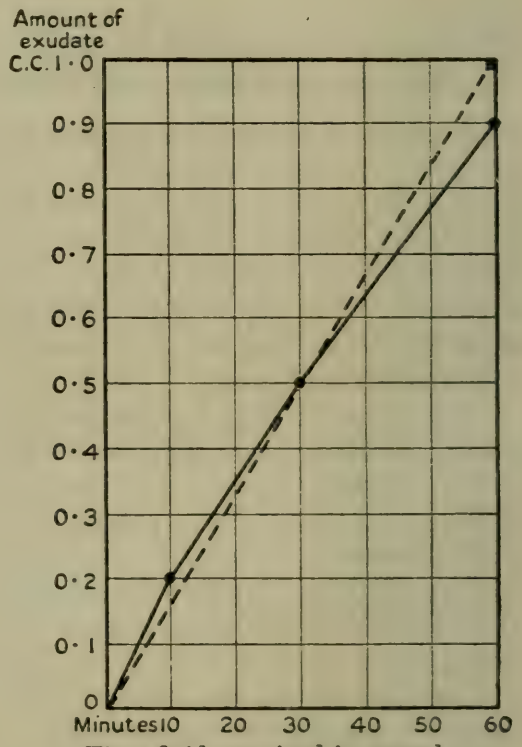
exudate. For instance, the 5-minute specimen was slightly mucoid. this being due to the action of the antiseptic on the leucocytes. The 10-minute specimen was mucoid and slightly red in colour. The 20-minute specimen was thick, mucoid and red, while the 30-minute specimen was almost a jelly and stained deeply red. This red colour was due to red blood-corpuscles, the presence of which indicated a marked irritant action of chloramine T in 4 per cent. solution.

(2) This experiment was carried out in the same manner on the wound ' P ', a 1 per cent. solution of the antiseptic being used.



Time fluid remained in wound.

Fig. XXXV. Rate of exudation into a wound filled with chloramine T (4 per cent.). Continuous line represents amount of exudate into cavity filled with chloramine T. Dotted line represents amount of exudate into empty cavity on the same day. Shaded area indicates the amount of exudate due to chloramine T.



Time fluid remained in wound.

Fig. XXXVI. Rate of exudation into a wound filled with chloramine T (1 per cent.). Continuous line = exudate into cavity filled with chloramine T. Dotted line = exudate into empty cavity on same day.

Fig. XXXVI shows that the amount of exudate passing into the solution was practically identical with that into the empty cavity. The irritant action on the walls of the wound was, in this case, entirely absent.

Experiments with Dakin's solution. (1) This was carried out as above but on the wound ' C '—and, in this case, the rate of exudation into the wound after withdrawal of the antiseptic was also estimated. The result is shown in Fig. XXXVII.

This Fig. shows that during the application of Dakin's fluid for the first 5 or 10 minutes the rate of flow is identical with that into the empty cavity. When the Dakin's solution had been in the cavity 20 minutes the exudate was slightly increased ; this was also the case after 40 minutes' application. When the fluid has

been removed the flow in the next 40 minutes is again slightly increased.

(2) Dakin's solution was applied to the same wound for 75 minutes. Fig. XXXVIII shows the rate of exudation into the wound before, during, and after the application. It will be seen that not only was the exudate increased during the application, but continued in excess of the normal rate for at least 7 hours after the antiseptic had been withdrawn. After 24 hours it had, however, returned to the normal amount.

(3) The wound was filled in with Dakin's fluid four times in 4½ hours. The fluid was changed after 1, 2, 3, and 4½ hours. After the last sample had been withdrawn the flow of exudate into the empty cavity was estimated after 1 and 2 hours' interval. The results are shown in Fig. XXXIX.

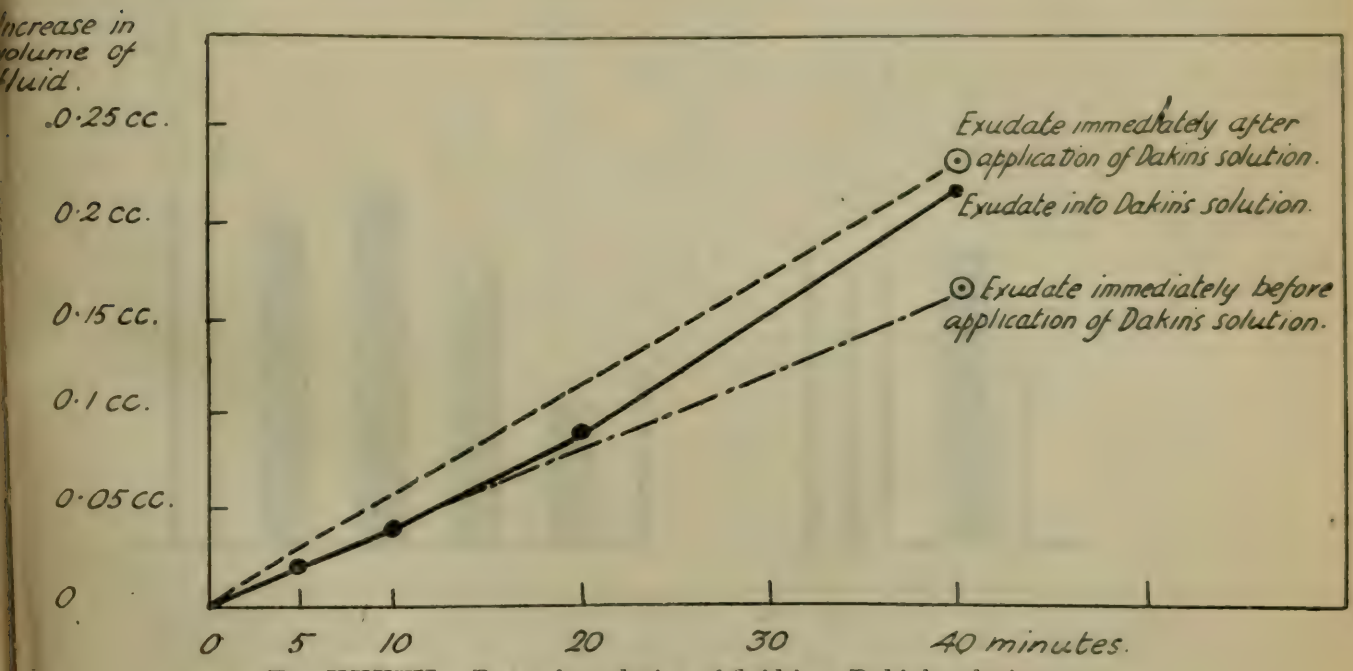


FIG. XXXVII. Rate of exudation of fluid into Dakin's solution.

This Fig. shows again that Dakin's fluid tends to increase the exudate after it has acted on the wound for a certain time ; in the present experiment it was slightly less after one hour, markedly increased after 2, 3, and 4 hours' application. Further, it shows that this increased flow continues after the removal of the fluid for at least 2 hours.

The above experiments indicate that Dakin's solution, after being applied to a wound surface, will tend to increase the flow of exudate into the wound, and this increased flow is continued for a considerable time after the Dakin's fluid has been removed.

The cause of this increased flow of exudate appears to be the result of an irritative action of Dakin's fluid, as there is at first a period during which the rate of flow is unaltered, and it is only later that the increase becomes marked.

This effect is exactly the reverse of that obtained with hypertonic salt solution, the application of which to the wound causes a maximal

increase of the exudate within a few minutes—as will be shown later.

Experiments with eusol. (1) In an experiment carried out on wound 'P' the rate of flow was measured on four occasions. The total period during which the eusol was applied to the wound was 30 minutes, and during that time it was changed on three occasions, namely, after applications of 5, 10, and 20 minutes.

These results—as well as the content of albumen in each sample of fluid—are shown in Fig. XL.

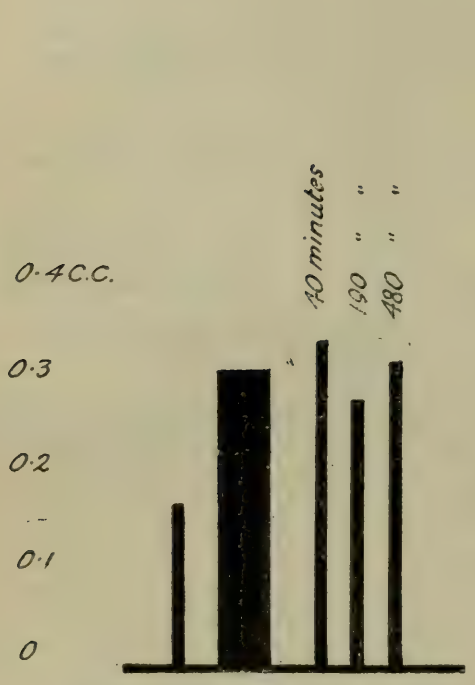


Fig. XXXVIII. Rate of exudation of fluid into Dakin's solution. The *thick* column represents the rate of flow of exudate per hour into the Dakin's solution. The *thin* column to the left represents the rate of flow per hour into the empty cavity *before* the application of the Dakin's solution. The thin columns on the right represent the rate of flow per hour into the empty cavity *after* the removal of the Dakin's solution.

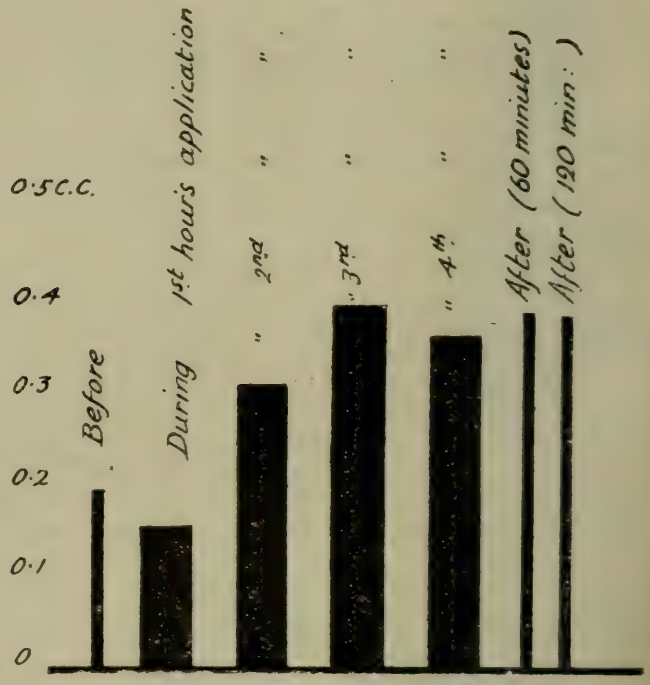


Fig. XXXIX. Rate of flow of exudate into wound before, during, and after the application of the Dakin's solution. The *thick* columns represent the rate of flow of exudate per hour into the Dakin's solution. The *thin* column to the left represents the rate of flow per hour into the empty cavity *before* the application of the Dakin's solution. The *thin* columns to the right represent the rate of flow per hour into the empty cavity *after* the removal of the Dakin's solution.

On examining this Fig. it will be seen that there was practically no flow after the first 10 minutes' application, while during the next 10 minutes (a total of 20 minutes) there was a rapid rate of flow into the cavity. During the final 10 minutes of the experiment the flow continued to be rapid.

(2) The wound 'C' was filled in with eusol, which was changed four times during the following 75 minutes, after which the wound was emptied and the rate of flow of exudate measured after periods of 40, 115, and 185 minutes. The rate of flow was also ascertained before the eusol was applied.

In the Fig. the results are shown in terms of rate of flow per hour. On examining this Fig. it will be at once noticed that during

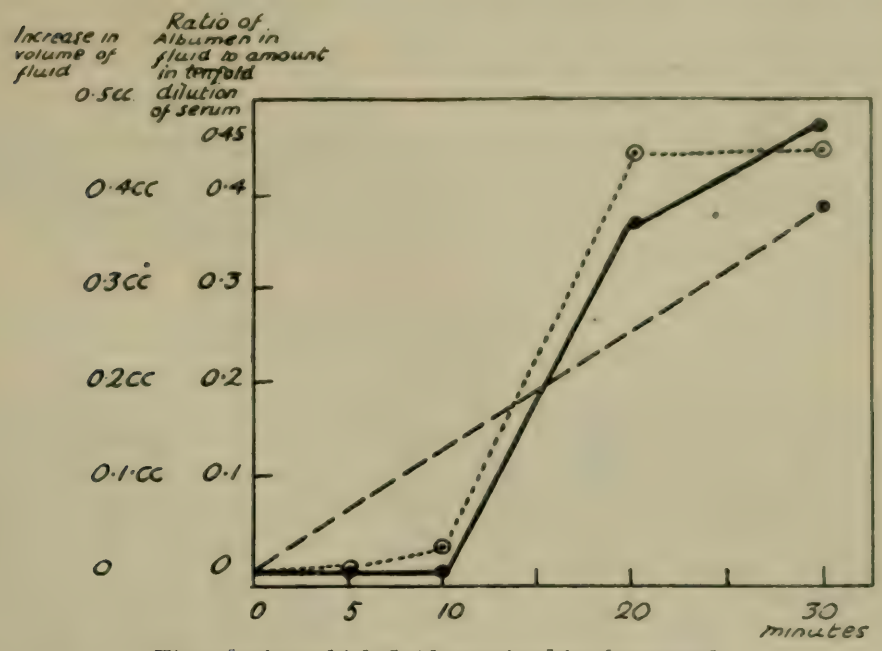


FIG. XL. Rate of exudation of fluid into eusol and amount of albumen in the exudate. Continuous line represents the rate of flow. Dotted line represents the amount of albumen. Interrupted line represents the average rate of flow into empty cavity on the same day.

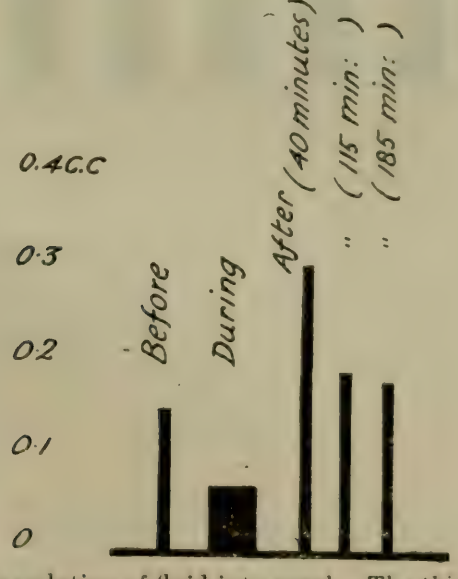


FIG. XLI. Rate of exudation of fluid into eusol. The *thick* column represents the rate of flow of exudate per hour into the eusol. The *thin* column to the left represents the rate of flow per hour into the empty cavity *before* the application of the eusol. The *thin* columns to the right represent the rate of flow per hour into the empty cavity *after* the removal of the eusol.

the application of the eusol the amount of exudate passing into the wound was distinctly decreased. A similar decrease was found to occur during the first 10 minutes in experiment 1. The explanation of this fact is not readily forthcoming, but it may possibly be due

to the action of the eusol causing some precipitation on to the walls of the wound cavity.

It will be seen that after the eusol had been removed from the wound the rate of flow was distinctly and markedly increased during the first 40 minutes, and was slightly in excess of the normal rate even after an interval of 3 hours.

Experiment with flavine. The wound 'C' was filled in with a 1 in 1,000 solution of flavine 5 times within 23¼ hours—the changes being made after 45 minutes, 1 hour, 2 hours, 4 hours, and 15½ hours. After the last sample had been withdrawn the exudation into the cavity was estimated at intervals as shown in Table XXXII.

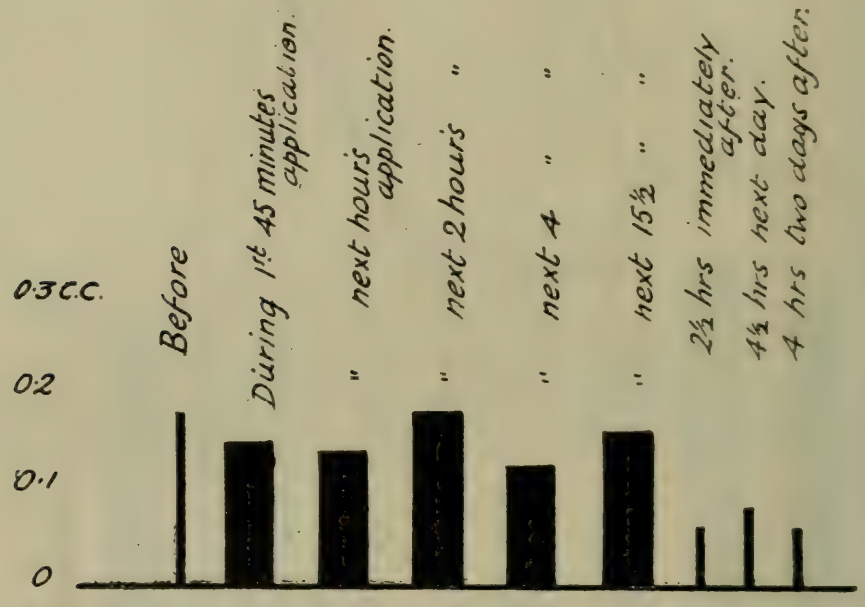


FIG. XLII. Rate of flow of exudate per hour into the wound before, during, and after the application of flavine solution 1 in 1,000.

TABLE XXXII.

Time, conditions, &c., under which the exudate was measured.	*Volume of exudate.*	*Rate of flow per hr.*
Flow into empty cavity 50 min. *before* introduction of flavine	0·15 c.c.	0·18 c.c.
Flow into flavine solution during first 45 min. . . .	0·11 c.c.	0·15 c.c.
" " " " following 60 min. . .	0·14 c.c.	0·14 c.c.
" " " " " 2 hrs. . .	0·38 c.c.	0·18 c.c.
" " " " " 4 hrs. . .	0·5 c.c.	0·125 c.c.
" " " " " 15½ hrs. . .	2·5 c.c.	0·16 c.c.
Flow into empty cavity during first 2½ hrs. after removal of flavine	0·14 c.c.	0·06 c.c.
Flow into empty cavity during 4½ hrs. on day following removal of flavine	0·37 c.c.	0·08 c.c.
Flow into empty cavity during 4 hrs., two days after removal of flavine	0·25 c.c.	0·06 c.c.

From these results it will be seen that, after the application of flavine to the wound for 23½ hours, the amount of exudate into the empty cavity was reduced to less than half the amount, and this continued for at least two days after the removal of the solution.

During the later period of the application of the flavine a thick

film of what may be described as sputum-like material was deposited on the walls of the wound cavity. This appeared to have but little inhibitory effect on the passage of fluid from the walls into the cavity as long as the flavine solution remained in the wound. When, however, the solution was removed, this membrane appeared to form a barrier to the passage of fluids, so that less than half the amount of exudate passed into the empty cavity.

The nature of the sputum-like material will be discussed more fully when dealing with the emigration of leucocytes.

These results are shown in graphic form in Fig. XLII.

(*b*) The Albumen Content of the Exudates Induced by the Presence of Antiseptics.

This was investigated only after the application of eusol and Dakin's solution respectively to the wounds 'P' and 'C'. The amount of albumen in the several samples of fluid was estimated by precipitation with salicylsulphonic or trichloracetic acids and subsequent centrifugalization in tubes of equal bore. The results were expressed as percentages of the precipitate obtained with a ten-fold dilution of serum. It was shown by each experiment—and is illustrated in Fig. XL—that increase of the amount of albumen in the fluid was roughly proportional to the increase of the exudate. This finding is in harmony with the conception that the increased exudation induced by the antiseptics is due to an irritation of the tissues—that it is, in fact, an inflammatory manifestation.

(*c*) The Number of Leucocytes in the Exudates.

This was determined by counting in an ordinary haemocytometer cell, the fluid being first diluted, in most cases, with a 1 per cent. solution of acetic acid tinted with neutral red. The control observation in these experiments was obtained by determining the number of leucocytes emigrating into normal salt solution.

Experiment with chloramine T, 1 per cent. solution. The number of leucocytes passing into the fluid is shown in the adjoined table

Table XXXIII.

Time one per cent. chloramine T solution had been in wound.	*Number of leucocytes per cubic millimetre of antiseptic + exudate.*
20 minutes	91,200
30 „	336,000
50 „	742,000

On comparing these figures with those in Table XXXV, which shows the result when normal saline solution was employed, it is seen that after 20 minutes this concentration of chloramine T does not hinder the emigration of leucocytes.

Experiment with Dakin's solution. The results are given in the following table :

TABLE XXXIV.

Length of time Dakin's solution had remained in the wound.	*Number of leucocytes per cubic millimetre of antiseptic + exudate.*
5 minutes	190
10 „	480
20 „	520
40 „	4,770

These results appear to indicate that Dakin's solution inhibits the emigration of leucocytes as long as the concentration of hypochlorite remains above a certain level, and this supposition is confirmed by a series of experiments *in vitro* which showed that the solution, in dilutions up to 1 in 32, completely inhibited the emigration of leucocytes into a fibrin network. These experiments would indicate that in 40 minutes the hypochlorite concentration of the fluid contained in the wound had fallen below even this figure.

Experiment with eusol. In samples of eusol withdrawn from the wound cavity after 5 and 10 minutes' application it was found to be quite impossible to make even an approximate count of the leucocytes which had been exuded from the walls of the wound, owing to the lytic action of the hypochlorite. Samples, however, removed after 20 minutes were found to contain, in the case of wound 'P', 350,000 leucocytes per c.mm., and after 60 minutes, 480,000 per c.mm. These figures correspond closely to those obtained when normal salt solution was employed in place of eusol, and this later emigration of leucocytes appeared to take place when the hypochlorite, as is shown in section (4), p. 79, had been reduced to a negligible concentration through its interaction with the exudate and wound walls.

Experiment with flavine, 1 per cent. solution. The number of leucocytes passing into the flavine solution gradually fell from 4,350 per c.mm. which were present after the solution had been in the wound for one hour to 850 per c.mm. in the specimen remaining in the wound for 4 hours. In the specimen which had remained in the wound for 15½ hours it was found impossible to make even an approximate count, as after that period the wound had become lined with the sputum-like membrane which was found to consist of leucocytes in various stages of degeneration. After the solution had been removed very few leucocytes passed into the wound cavity for at least two days.

(*d*) THE ANTITRYPTIC VALUE OF THE EXUDATES.

Wright's work[1] has emphasized the importance of the antitryptic power of the blood fluids and wound exudates in relation to microbic infection. The effect of eliminating this quality of the serum by additions of trypsin was well shown by experiments in which the amount of microbic growth was measured by gas formation as described in section (5). Fig. XLIII illustrates an experiment of this kind in which the two series of tubes contained infected serum whose antitryptic power was progressively diminished (from right to left) by increasing additions of trypsin. In the three or four tubes on the left of each series the serum thus treated is comparable to the 'corrupted lymph' which is present in every infected wound in which

[1] Wright, *Lancet*, 1915, i, 737, 787, 843, 897.

large numbers of leucocytes are being disintegrated (with liberation of tryptic ferment). The increased growth of the microbe in these tubes gives us warning as to the undesirability of permitting any considerable reduction in the antitryptic value of the wound exudates. Fig. XLIV illustrates the same point in connexion with the growth of *Streptococcus* in serum.

In reality the importance of the antitryptic power of the exudates is greater than even these experiments indicate—for not only does the fluid in which that quality is abolished become changed into a favourable culture medium, but its opsonic and other antibacterial actions are also lost.

It was with these considerations in view that the effect of antiseptics upon the antitryptic value of the exudates was investigated.

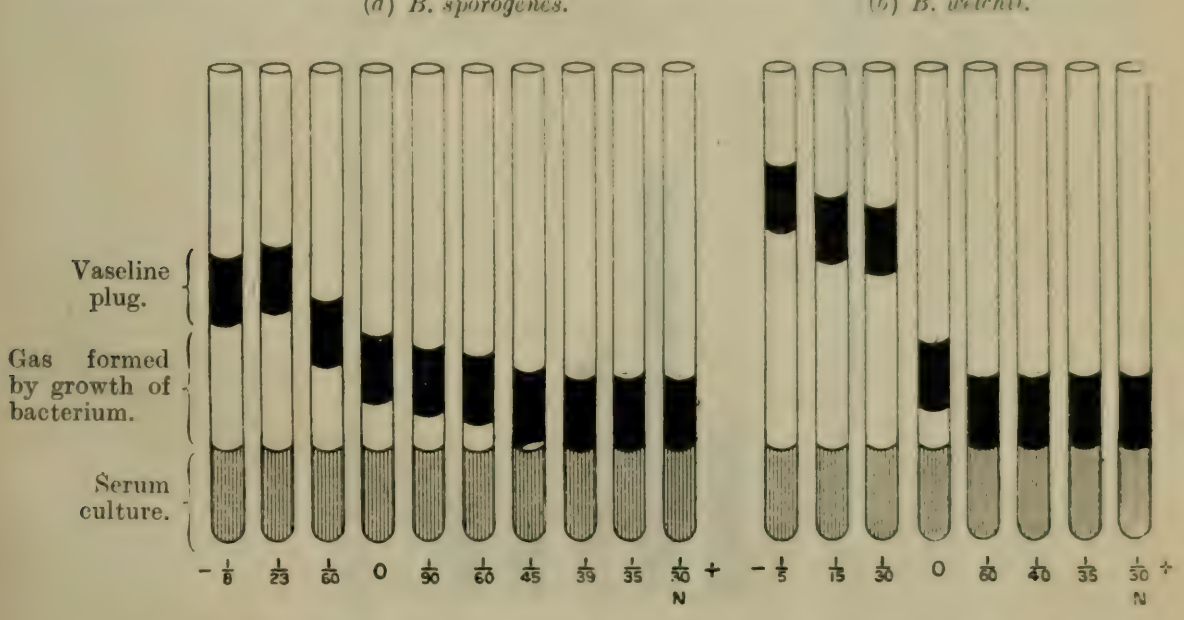


FIG. XLIII. Showing the effect of a reduction in the antitryphic power of the serum on the growth of (*a*) *B. sporogenes*, (*b*) *B. welchii*.

The figure given below each tube indicates the anti-tryptic, or, as the case may be, the tryptic power of the serum employed in that tube.

That labelled 'O' has had its antitryptic power exactly neutralized by the addition of trypsin. The tubes on the left of this one in each series contain free tryptic ferment in increasing quantities, while those on the right become increasingly antitryptic, until the one on the extreme right represents unaltered serum with its normal antitryptic power. (Equivalent to a 1 : 30 dilution of the trypsin used.)

Method of investigation. The fluid exuding into the wounds was collected before the application of the antiseptic, and at intervals after this had been withdrawn. It was first tested with a view to determining whether it contained free tryptic ferment. For this purpose a milk-clotting method, based on Mellanby's[1] work, was employed. One volume of the clear supernatant obtained by centrifuging the fluid was mixed with one volume of separated milk to which 0·5 to 1·0 per cent. of calcium chloride (cryst.) had been added. This mixture was drawn up into the stem of a capillary pipette and incubated at 50° C. for half an hour. A control mixture, in which the exudate was replaced by water, was also incubated,

[1] Mellanby and Woolley, *J. Physiol.*, 1912, xlv. 370.

in order to make sure that the milk had no tendency to clot spontaneously. If, at the end of the incubation, the milk mixed with exudate had clotted, while that in the control tube remained unclotted, the exudate was judged to contain free tryptic ferment.

Failing this result, it was necessary to proceed to a further test with a view to determining the *antitryptic* power of the exudate. For that purpose a series of tubules was filled in with (*a*) one volume of the exudate to be tested, (*b*) one volume of a series of progressive dilutions of a trypsin solution, (*c*) two volumes of milk to which 1 per cent. of calcium chloride had been added. The tubes were then placed in a water bath at 50° C. for half an hour and examined to ascertain in which tubes clotting of the milk had taken place. The antitryptic power was then expressed in terms of the strength of the trypsin solution which was just neutralized by the fluid.

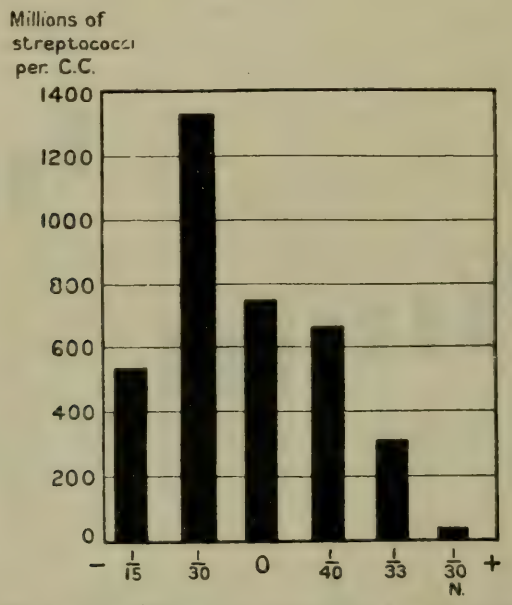


Fig. XLIV. Showing the effect of a reduction in the antitryptic power of the serum on the growth of *treptococcus*.
For explanation of the figures below each column see description of Fig. XLIII.

Experiment with Dakin's solution. The exudate was tested before and at intervals of 1 and 2 hours after withdrawal of the solution from the wound 'C'—in which it had been allowed to act for 4½ hours. The result is shown in Fig. XLV.

From this chart it will be seen that for at least 2 hours after the Dakin's fluid had been removed there was a very considerable drop in the antitryptic power of the serum. In connexion with this effect it may be recalled that the application of Dakin's solution has frequently been accredited with hastening the separation of sloughs in infected wounds.

Experiment with eusol. The wound 'C' was treated for 75 minutes with eusol, and the exudate collected at intervals of 40, 115, and 185 minutes after its removal, as well as before the application. The results are shown in Fig. XLVI.

On examining this Fig. it will be seen that during the first 40 minutes after the eusol had been removed the antitryptic power of the

exudate was very much diminished, after 115 minutes it had recovered to some extent, but even after 185 minutes it was still less than before the application of the eusol. This diminished antitryptic power is most probably due to the breaking down of leucocytes and other cells in the walls of the wound, in consequence of which the trypsin contained is set free and partially neutralizes the antitryptic power of the serum.

Experiment with flavine, 1 *in* 1,000 *solution*. This antiseptic was

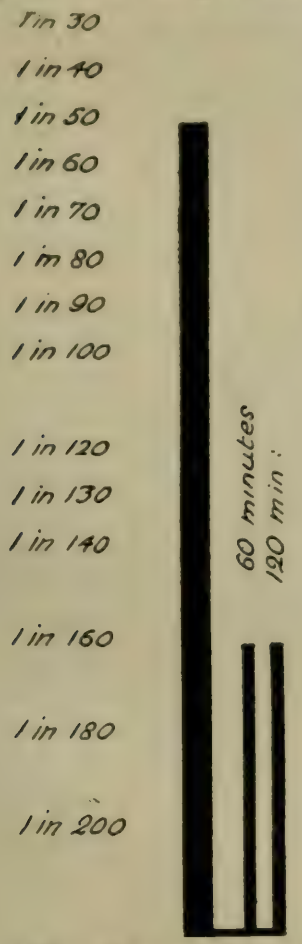


FIG. XLV. *Effect of Dakin's solution on the antitryptic power of the exudate.* Dilution of trypsin neutralized by an equal volume of the exudate. The *thick* column indicates the antitryptic power of the exudate into the empty cavity *before* the application of the Dakin's solution. The *thin* columns indicate the antitryptic power of the exudate into the empty wound at intervals *after* the removal of the Dakin's solution. The times are given above the columns.

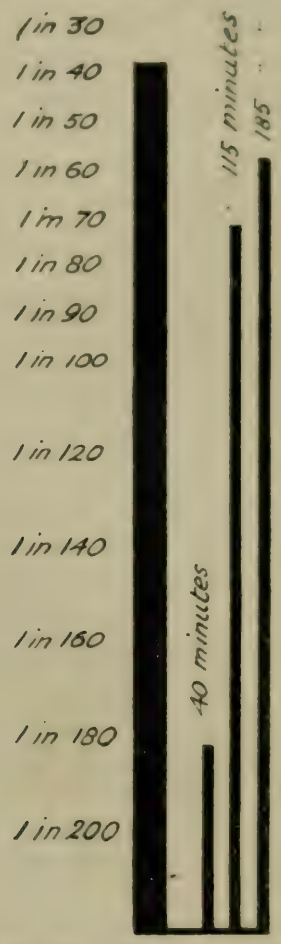


FIG. XLVI. *Effect of eusol on the antitryptic power of the exudate into the wound.* Dilution of trypsin neutralized by an equal volume of the exudate. The *thick* column indicates the antitryptic power of the exudate into the empty cavity *before* the application of the eusol. The *thin* column indicates the antitryptic power of the exudate into the empty cavity at intervals *after* the removal of the eusol. The times the samples were taken are given above the corresponding columns.

applied five times in the course of 24 hours to the wound [illegible]. The exudates collected before the application and 2½ hours after the solution had been removed were found to have the same antitryptic power. The result is somewhat surprising in view of the marked effect which a prolonged application of flavine has been shown to have on the leucocytes, massing them into a tenacious mucoid-like membrane adhering to the walls of the wound; it is not improbable,

however, that their contained trypsin remains bound to the sputum-like material as is the case in pneumonic sputum which similarly consists largely of leucocytes broken down into a mucoid-like material.

(11) Experiments bearing upon the employment of hypertonic salt solutions in the treatment of infected wounds.

As is now well known, Wright has recommended as a treatment for suppurating, slough-covered wounds, the application of a 5 per cent. solution of sodium chloride, with a view to (*a*) the rapid separation of sloughs by the agency of tryptic ferments, which he showed to be set free by the action of the solution on leucocytes, and (*b*) the production of an increased flow of lymph, which, by virtue of its bacteriotropic properties, should kill off, or inhibit the growth of, many of the bacteria contaminating the wound and surrounding tissues.

He further recommended that, when the wound had become clean under this treatment, the hypertonic solution should give place to physiological salt solution (0·85 per cent.), the aim then being to obtain an abundant emigration of leucocytes into the wound and by means of these to achieve the destruction of such serophytic organisms as had not been eliminated from the wound by the increased lymph flow.

To these recommendations certain objections had been raised; it was put forward that the conditions of the experiments *in vitro* on which Wright based his teaching were not similar to those obtained in wounds—and, in particular, it was contended that the increased flow, caused by the drawing action of 5 per cent. salt solution, would consist, not of lymph, but of fluid containing little or no albumen.

To see how far experiments *in vivo* would bear out Wright's contentions and in particular to obtain evidence as to the nature of the fluid drawn out by the salt solution, a series of observations similar in kind to those already described in connexion with the investigation of antiseptic solutions was made on the cup-shaped wounds 'P' and 'C'.

The data which we obtained may be set out under the following heads:

(*a*) The effect of hypertonic salt solution on the rate of exudation into the wound.

(*b*) The albumen content of the exudation induced by salt solution.

(*c*) The number of leucocytes present in such an exudate, and also in that which takes place into physiological salt solution.

(*d*) The effect of hypertonic salt solution upon the antitryptic value of the exudate.

(*a*) The Rate of Exudation.

Experiment 1. The normal rate of flow into the wound 'P' having been determined, it was filled in with 5 per cent. salt solution, and measurements of the contents were made at frequent intervals. These were plotted out in Fig. XLVII.

Experiment 2. The flow into wound 'C' was estimated at intervals: (1) when the cavity was empty; (2) when the cavity was filled with

physiological salt solution; and (3) when it was filled with 5 per cent. salt solution. The results are shown in Fig. XLVIII.

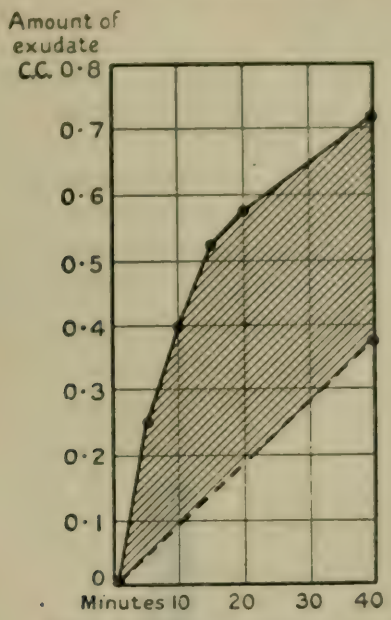


FIG. XLVII. Rate of exudation of fluid into a wound cavity filled with 5 per cent. salt solution. The continuous line represents the amount of exudation. The dotted line represents the amount of exudation into the empty cavity of the wound on the same day. The shaded area indicates the amount of exudation which is actually due to the salt solution.

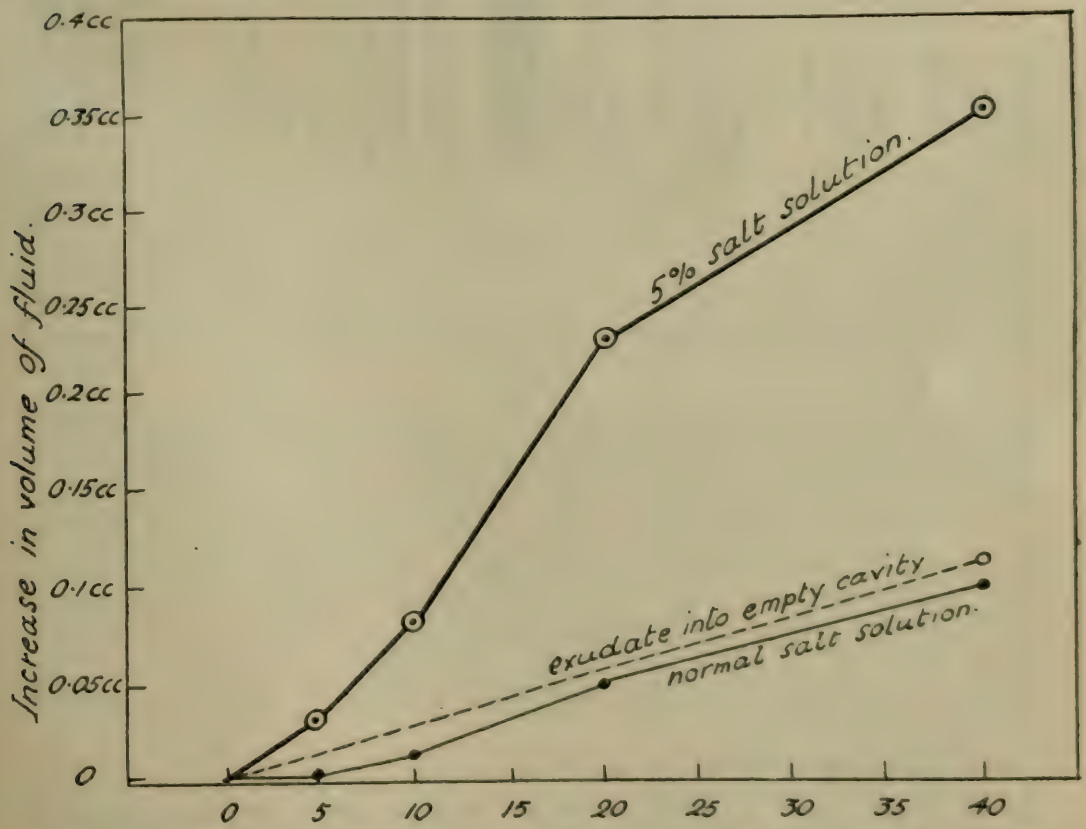


FIG. XLVIII. Rate of exudation of fluid into a wound cavity (*Experiment* 2): (*a*) when empty; (*b*) when filled with normal salt solution; (*c*) when filled with 5 per cent. salt solution.

It will be seen from this Fig. that the flow into 0·85 per cent. salt is practically the same as that which takes place into the empty cavity, but that, as in Experiment 1, a much greater flow was induced by the 5 per cent. solution.

Experiment 3. Five per cent. salt solution was left in the wound for 75 minutes. The amount of exudate before, during, and after this application is shown in Fig. XLIX.

The new point brought out by this experiment is that the increased flow continued for at least 30 minutes after withdrawal of the salt solution ; and further, by estimating the salt content of the several exudates, it was ascertained that the fluid passing into the cavity after removal of the salt solution was considerably richer in sodium

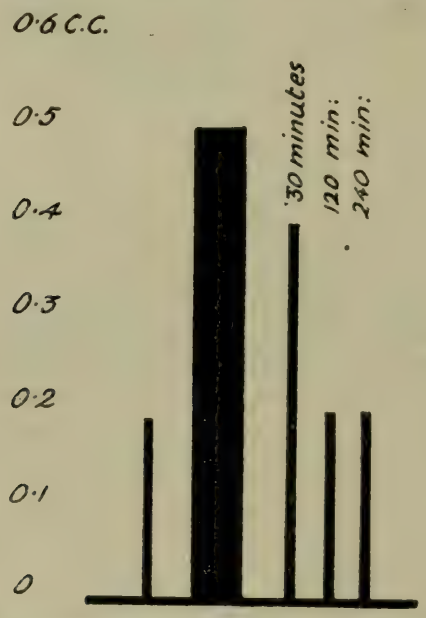


Fig. XLIX. *Rate of flow of exudate into the wound before, during, and after the application of 5 per cent. sodium chloride solution.* Increase in volume of fluid. The *thick* column represents the rate of flow of exudate per hour into the sodium chloride solution. The *thin* column to the left represents the rate of flow per hour into the empty cavity *before* the application of 5 per cent. sodium chloride solution. The *thin* columns to the right represent the rate of flow per hour into the empty cavity at different periods *after* the removal of the 5 per cent. sodium chloride.

chloride than the normal exudate. The increased flow was most probably due to the passage outwards of the sodium chloride which had previously passed into the tissues from the 5 per cent. solution.

In concluding this part of the investigation comparison may be made between the amount of exudation induced by salt solution and that which was obtained by the application of certain antiseptics to the same wound. For this purpose Figs. XXXVIII, XXXIX, XLI, XLII, and XLIX are here reproduced in combination, see Fig. L. It will be seen that the effect exerted by salt solution is by far the greatest.

Reduction of strength of the salt solution in the wounds. Experiments 1 and 2 above showed that the increase of the exudate induced by the presence of hypertonic salt solution in the wound was most marked when this solution was renewed at frequent intervals. This result was, of course, to be expected, for it will be evident that the solution in the wound will progressively diminish in strength, both by dilution

with the incoming lymph and by diffusion of the sodium chloride into the tissues.

Experiment 4 was carried out to measure the diminution in the strength of the solution. The wound 'P' was filled in with a 5 per cent. solution which remained for varying periods, e. g. 5, 10, 15 minutes, &c., as shown in Fig. LI. The concentration of salt in each sample was estimated as follows. A suspension of human red blood corpuscles thoroughly washed free from serum was made in 0·85 per cent. sodium chloride solution. Twenty c.mm. of this suspension was then mixed with 20 c.mm. of the fluid which had been in the wound for a known length of time, rendered clear by centrifuging. Distilled water was now carefully added in sufficient quantity to cause complete haemolysis of the suspended corpuscles. The amount of distilled water necessary to produce haemolysis was carefully noted. A control series of experiments was then made in which, in place of

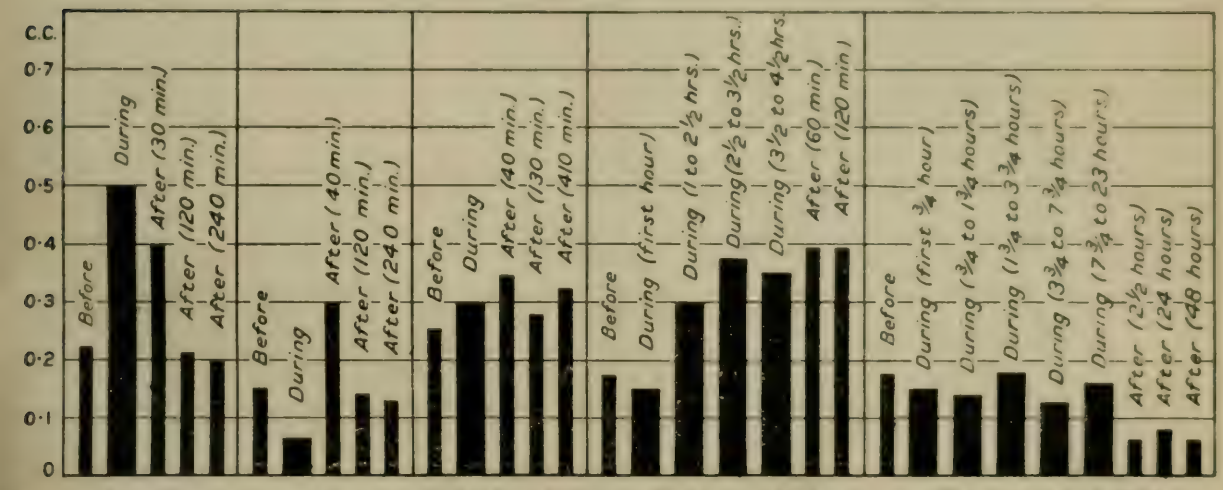


Fig. L. Transudation of fluid per hour into a wound cavity before, during, and after the application of salt solution, eusol, Dakin's solution, and flavine.

the fluid which had been in contact with the wound, known strengths of salt solution were added to the suspension of corpuscles; the amount of distilled water necessary to cause complete haemolysis in these mixtures was then estimated. By comparing the amount of distilled water used in the two series the concentration of salt in the various samples was easily estimated. This method was suggested by Wright's technique[1] for estimating the amount of salt in the blood and urine.

Using this technique, it was found that the rate at which salt disappeared from the solution in the wound was much more rapid in the early stages of the experiment than in the later ones; for instance, in the first 10 minutes of the experiment the concentration fell from 5 per cent. to 3·01 per cent., a loss approximately of 2 per cent., while the difference between samples remaining in the wound 40 and 60 minutes respectively was only 0·36 per cent. (1·93 to 1·57 per cent.). After the solution had remained in the wound for 60 minutes the concentration had fallen to 1·57 per cent.

The results are shown in Fig. LI.

[1] Wright, *Technique of the Test and Capillary Glass Tube*, London (Constable), 1912, 97.

(*b*) The Albumen Content of the Exudate Induced by Salt Solution.

This was estimated as described on p. 107. The wound 'C' was filled in for varying periods with 0·85 per cent. salt solution, and later with a 5 per cent. solution.

Fig. LII shows quite distinctly that the amount of albumen in solution which passed from the walls of the wound into the 5 per cent. solution was much in excess of that which passed into normal saline solution.

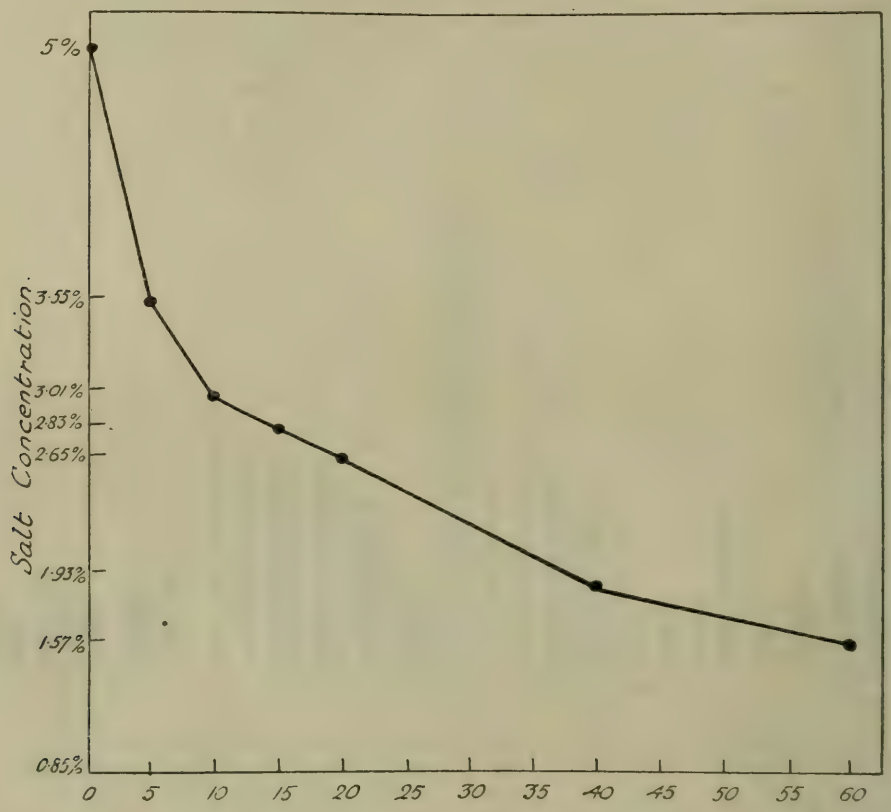


Fig. LI. Rate of diminution of strength of 5 per cent. salt solution left in a wound.

(*c*) The Number of Leucocytes Contained in the Exudate.

This was investigated on several occasions when the fluid used in filling in the wounds was respectively normal saline solution and 5 per cent. sodium chloride solution. The results are given in the following table:

Table XXXV.

Time during which the fluid had remained in the wound.	Wound 'P'.		Wound 'C'.	
	Normal salt.	*5 per cent. salt.*	*Normal salt.*	*5 per cent. salt.*
5 minutes	36,000	16,400	5,160	1,400
10 ,,	78,000	36,400	16,600	1,440
20 ,,	346,000	62,000	20,000	1,660
30 ,,	434,000	125,600	..	—
40 ,,	..	..	28,000	2,000
60 ,,	593,000	234,000	..	..

This table shows conclusively that the leucocytes pass out much more readily from the granulation tissue into the cavity of the wound when this contains normal saline solution than when it contains 5 per cent. sodium chloride solution. It is also probably true that in the presence of normal saline solution the passage of the leucocytes is greater than when the cavity is left empty ; this, however, could not be estimated with sufficient accuracy, owing to technical difficulties, for definite figures to be given.

(*d*) The Effect of Hypertonic Salt Solution upon the Antitryptic Value of the Exudate.

No observations were made directly upon the liberation of tryptic ferments by the action of 5 per cent. salt solution on the

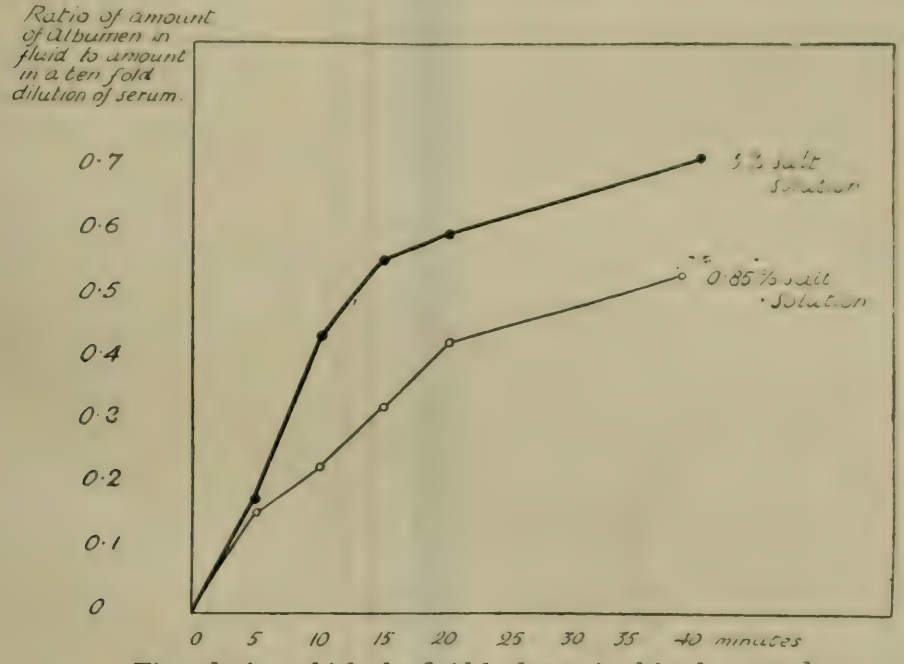


Fig. LII. Showing the amount of albumen present in the exudate when the wound was filled with 5 per cent. and 0·85 per cent. salt solution respectively.

leucocytes. The only experiment we carried out was for the purpose of testing whether the lymph passing into the wound after withdrawal of the salt solution had a diminished antitryptic power.

Fig. LIII shows that for half an hour a slight diminution did occur, but that after 2 hours the antitryptic power was the same as before the application of the salt.

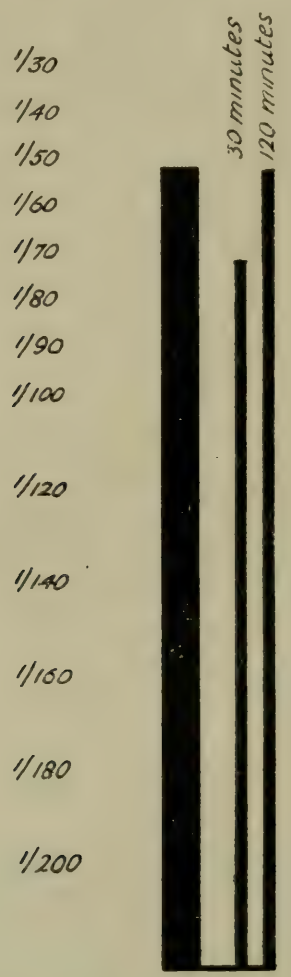
(12) Summary and discussion of results.

(1) Two simple cup-shaped wounds have served us as 'test tubes' by means of which the action of certain antiseptics and of hypertonic salt solution—as well as the reactions of the tissues to these substances—could be studied *in vivo*.

(2) The application of eusol, Dakin's solution, and solutions of chloramine T to these wounds, in such a manner as to give the

antiseptic the best possible chance of exercising its bactericidal effect, caused no appreciable reduction in the bacterial flora of the wounds (vide Section 3). Owing to technical difficulties, the analogous experiment with flavine did not give a clear result, but it can be said that nothing like a complete bactericidal effect was obtained.

(3) The effective strength of eusol and Dakin's solution is very rapidly dissipated by contact with the tissues when applied even to



The times are given above the columns.

FIG. LIII. *Effect of 5 per cent. salt solution on the antitryptic power of the exudate* The *thick* column indicates the antitryptic power of the exudate into the empty cavity *before* the application of the 5 per cent. salt solution. The *thin* columns indicate the antitryptic power of the exudate into the empty cavity at intervals *after* the removal of the 5 per cent. salt solution.

a perfectly clean wound. Within 10 minutes their hypochlorite concentration is reduced by at least 80 per cent. and their bactericidal value has become practically nil. Very much the same applies to chloramine T. (It is probable that a still greater loss of strength would have been recorded if these antiseptics had been applied to wounds in which there was a large accumulation of leucocytes, a condition which frequently obtains in surgical practice and, especially, when Carrel's system of intermittent irrigation is employed.)

In the case of flavine a similar but slower reduction in strength

occurs as the dye becomes combined with the tissues of the wound; and in this case—as with all other dyes tested—a further serious loss of effective strength occurs through fixation of the dye by the cotton and other fabrics which are habitually employed for dressing wounds.

(4) In view of these serious drains upon the effective strength of antiseptics in a wound, the question naturally arose—Is there a sufficient amount of the antiseptic agent left in the wound to exercise a bactericidal effect? This problem was investigated by determining the effect of various strengths of antiseptic solutions upon bacteria suspended in serum. In the case of eusol and Dakin's solution it was found that a hypochlorite concentration, comparable to that which remains in a wound 5 minutes after its application, was not only incapable of restraining the growth of microbes in serum but actually *stimulated* the growth of certain types to a very marked degree. In the case of flavine, concentrations of 1 in 4,000 to 1 in 16,000 were found to be necessary to inhibit the growth of *Staphylococcus*, according to the number of cocci employed for the test—and it appears unlikely that such concentrations as this remain active in the wound for any length of time after the application of a 1 in 1,000 solution.

The stimulation of microbic growth in serum was not confined to the hypochlorite solutions, being demonstrated also with carbolic acid, iodine, chloramine T, and malachite green solutions. It did not occur equally with all bacteria.

(5) In Section 9 it is shown that all the antiseptics in use have a destructive action on the leucocytes, and this destructive action occurs in a lower concentration than is necessary for a lethal action on the bacteria. Wright, Fleming, and Colebrook[1] have demonstrated the very striking bactericidal efficiency of leucocytes when provided with the requisite conditions for their functioning. In the cavity of a wound to which an antiseptic has been applied such leucocytic destruction of microbes will be put out of action for a time varying with the rate of dissipation of the antiseptic, and in this way it may again happen that the balance of advantage, following the employment of such an agent, will be with the bacteria rather than the patient.

(6) It has been suggested that certain antiseptics, although incapable of exerting a directly bactericidal effect in the wound, may yet contribute something indirectly to the antibacterial processes by provoking a *physiological reaction* on the part of the tissues. It has also been stated as a fact of clinical observation that the separation of sloughs in infected wounds is hastened by the use of Dakin's solution. In this connexion our experiments showed that the application of hypochlorite solutions resulted in an increased exudation of lymph, which sometimes lasted over a period of several hours—and also that this lymph differed from that normally passing into the wound in that its antitryptic power was much reduced. This latter result may help to explain how the antiseptic has promoted (*sic*) the separation of sloughs. In our experiments—which were done with clean wounds—its application led merely to a diminu-

[1] Wright, Fleming, and Colebrook, *Lancet*, 1918, i. 831.

tion of the antitryptic power of the exuded lymph (presumably by disintegration of leucocytes), but in a foul wound full of dead leucocytes, it is probable that the same process would make the discharges actively tryptic, or increase the amount of tryptic ferment already liberated in the wound, and thus lead to the digestion of sloughs.

In a wound already free from sloughs, on the other hand, it would seem that no useful purpose can be served by the increased flow of lymph of reduced antitryptic power obtained by this means. Wright has shown that what is required in such a wound is not so much a flow of lymph as an abundant emigration of leucocytes and the maintenance of optimum conditions for their functioning. The use of antiseptic solutions is directly opposed to these aims.

(7) The effects of introducing hypertonic salt solution into a wound were studied and found to be as follows :

(*a*) An immediate and marked increase in the exudation occurring into the wound cavity, this increase being greater than that obtained by the irritant action of any antiseptic solution.

(*b*) The exudate so obtained did not consist merely of watery fluid, as had been sometimes alleged, but was rich in albuminous substances.

(*c*) The emigration of leucocytes into the wound was suspended for a time but gradually returned to normal as the sodium chloride concentration of the fluid fell away. An abundant emigration of leucocytes took place when the wound contained physiological salt solution.

These findings agree in every particular with the results of experiments *in vitro* published by Wright in the early days of the war. He also demonstrated very clearly that the disintegrative action of 5 per cent. salt solution upon the leucocytes of pus led to a liberation of tryptic ferment, and upon this basis advocated the frequent application of the solution in the treatment of slough-covered wounds.

Wright's work, together with the series of experiments here described, enable us to piece together the cycle of events occurring after the instillation of 5 per cent. salt solution into a wound, somewhat as follows :

First 20 minutes. Rapid exudation of lymph ; liberation of tryptic ferment by disintegration of leucocytes present on the surface of the wound, in sloughs, and free in the pus (this ferment, however, will not be able to exert its full digestive effect until the salt concentration has fallen considerably) ; emigration of fresh leucocytes almost entirely suppressed ; concentration of the salt solution falling roughly to one-half its original content.

Second 20 minutes. Less rapid exudation of lymph, but the antitryptic power has been abolished owing to the disintegration of leucocytes by the salt solution, which has now diffused into the walls of the wound ; digestion of sloughs by the tryptic ferment in the cavity of the wound becoming very active ; emigration of leucocytes recommencing ; concentration of salt solution falling further—to 1·5 or 2 per cent.

Third 20 minutes. Exudation again less abundant but still more

than normal owing to the high sodium chloride content of the tissues ; lymph distinctly tryptic ; digestion of sloughs proceeding at maximal rate ; emigration of leucocytes becoming more abundant; concentration of salt solution nearing the isotonic level.

Second hour. Little change in rate of exudation—the lymph less tryptic or becoming slightly antitryptic ; digestion of sloughs continuing but not quite so actively as before ; emigration of leucocytes at normal rate and some of them undergoing natural disintegration, so reinforcing slightly the tryptic ferment. As the wound becomes progressively cleaner the serous exudate will tend to have a greater antitryptic value and will, therefore, be enabled to exert its antibacterial properties upon any sero-saprophytic bacteria that may be present.

In addition to the investigations here recorded the Authors have reported elsewhere upon three subjects which arose out of the work, and particularly attracted their attention. As these papers are available for reference they will not—in view of the present heavy cost of printing—be here reproduced.

They are as follows :

1. *On the Question of Bacterial Symbiosis in Wound Infections. Lancet*, 1917, i. 604.

The findings of this paper indicate that symbiosis may play an important part in regard to the proliferation of bacteria in wounds.

The growth *in vitro* of pure cultures of the anaerobic bacteria commonly found in wounds is much less rapid and less abundant than is the case when either streptococci, staphylococci, diphtheroid bacilli, *B. proteus*, *B. pyocyaneus* or coliform bacilli are grown in association with them.

Similarly the growth of streptococci is greatly facilitated by association with diphtheroid bacilli. Diphtheroid bacilli and many other bacteria are shown to absorb oxygen during their growth, and it is suggested that the symbiotic effects obtained may be ascribed in part to that process.

2. *On the Growth of Anaerobic Bacilli in Fluid Media under apparently Aerobic Conditions. Lancet*, 1917, ii. 530.

The presence of some porous substance is shown to favour the growth of anaerobic bacteria in a fluid medium. Growth is shown to occur first in the interstices of such a substance where, presumably, the bacteria are able to create partially anaerobic conditions by absorption of the oxygen.

3. *On Skin Grafting. Lancet*, 1917, ii. 5.

Some 33 transplantation operations are reported—a large number of them being successful. Small grafts were employed and the raw surfaces from which they were taken were afterwards closed with sutures. Local anaesthesia (by nerve blocking) was usually employed instead of general anaesthesia. The importance of dressing the grafted wound daily is emphasized, and certain recommendations made with regard to the technique of dressings.

In the general treatment of the cases the authors had the advantage of being able to consult the Surgical and Medical Staff of St. Mary's Hospital, and they take this opportunity of recording their thanks to Mr. V. Warren Low, Mr. Clayton Greene, and Mr. Fitzwilliams for their advice and for the performance of some operations, and to Dr. Wilfred Harris for examining several cases of nerve injury. Their thanks are also due to Dr. M. M. Bird, the Resident Medical Superintendent, St. Mary's Hospital, who was kind enough to undertake the temporary charge of three patients whom it was thought desirable to isolate. They would thank Mr. G. V. Davis and Mr. B. Thomas who, during the period of the research, were house-surgeons to the wards of the Inoculation Department, and to Mr. T. M. Davis who, for some weeks, undertook these by no means light duties in addition to his other hospital work. They would also record with thanks the help received from Dr. D. G. Sutherland, in respect to carrying out some routine bacteriological investigations, and to the late Dr. J. B. Nias for his help in the wards, at a time when the work was more than usually arduous. Lastly, they wish to place on record their appreciation of the way in which the Sister-in-charge of the Inoculation Department Wards, Miss Winson, carried out her duties. Her devotion to the patients, quickness to grasp new methods, and the carrying out of many instructions foreign to her usual duties, greatly assisted the work.

APPENDIX

CLINICAL ACCOUNT OF THE 61 CASES, WITH BACTERIOLOGICAL NOTES

Case No. 1. Wounded (bullet) 1.7.16. Admitted 16.7.16. Discharged 26.9.16.

History. The bullet had entered the front of the right thigh about 4 inches above the patella, causing a fairly clean fracture of the bone, and making a large ragged exit wound. On July 4th, on his arrival at the Base hospital, the leg was found very septic, and incisions were made evacuating large quantities of pus; a quantity of sloughs and gangrenous tissue was also removed.

Condition on admission (16th day). The patient's general condition was fair. On examination, a very extensive wound was found on the posterior surface of the right leg, the skin and intermuscular tissue of the whole of the posterior aspect of the thigh being destroyed, except for a narrow bridge of skin from the gluteal fold to the middle of the calf. The muscles lay practically separated from one another. The femur was fractured between 5 and 6 inches above the knee and large quantities of pus were being discharged, especially from the seat of the fracture. The wound of entry had healed.

Treatment. The limb was splinted and intermittent irrigations of 5 per cent. salt solution were given. The local condition improved considerably, and granulations were formed, rapidly binding the muscles together and filling up the deeper parts of the wound, while a very rapid growth of epithelium took place, especially at the upper part of the wound. The average evening temperature was, at first, about 102·5° F., falling later under treatment with small doses of vaccine made from *M. tetragenus* and *Streptococcus* isolated from the wound to 101° F. In spite of all care a bed sore formed and the patient's general condition became gradually worse, so the leg was amputated through the middle of the thigh on August 11th by a racquet-shaped incision, no attempt being made to close the wound. Recovery was uninterrupted and very rapid, the stump being at first dressed with wet dressings of 5 per cent. saline, later with normal saline, the dressings being separated from the raw surface by a sheet of perforated oiled silk to prevent them sticking. Early extension was applied to the skin, and the whole wound was healed with a firm scar and well covered bone by September 25th (45 days after amputation).

Bacteriological Notes.

On admission, July 16th. *B. sporogenes*, *B. tertius*, *B. butyricus*. An anaerobic tetrad. *Streptococci* (liquefying faecalis type). Coliform and diphtheroid bacilli. *Staphylococci* (a few).

July 24th. Film only. Tetrads very numerous. *Streptococcus*, Gram-positive bacilli. Diphtheroids.

August 2nd. *B. sporogenes*. A coliform bacillus and *Streptococcus equinus*.

August 16th. *B. sporogenes*. *B. proteus* and principally *Streptococcus faecalis*.

August 18th. No anaerobes.

September 18th. *Streptococcus faecalis* and *B. proteus*.

Note. The anaerobes did not disappear from the wound as quickly as usual, a very few being found (*B. sporogenes*) as late as August 2nd. After the amputation they reappeared, and it may here be noted that a few days after any operation, unless the wound is practically sterile, the opening up of areas of uninfected tissues always leads to an immense increase of the bacterial flora. As regards the streptococci, it is noteworthy that this is one of the very few cases which remained throughout the whole of the course of treatment without *Streptococcus pyogenes* ever being found, and it is considered that the absence of this organism accounts for the extremely favourable way in which the amputation wound healed up.

Case No. 2. Wounded (shell) 11.7.16. Admitted 19.7.16. Discharged 27.1.17.

History. At the Casualty Clearing Station the wounds were opened up and much muscle was excised for gas gangrene.

Condition on admission (8th day). General condition good. There were two

extensive wounds on the calf of the right leg, one on the inner side, the other on the outer, with much loss of skin and muscle. The outer wound was roughly oval in shape, about 5 inches long by 4 inches broad, and included an extensive comminuted fracture of the fibula, of which bone a considerable portion was missing. The anterior tibial and peroneal nerves were completely divided. Much muscle tissue had been destroyed. There was a deep pocket passing from the lower portion of the wound downwards between the tibia and fibula. The wound on the inner aspect, which was separated from the outer one by a bridge of healthy tissue and skin, was roughly oval, being about 6 inches long and 4 inches broad, and extended into the calf muscles.

Treatment. With intermittent application of hypertonic salt solution the wound surfaces rapidly became clean and healthy looking but the pocketing was very troublesome, and the temperature remained high until small doses of streptococcus vaccine were given, when it rapidly became normal.

In connexion with this case observations were made as to the stimulant action of malachite green on the growth of epithelium. A 1 per cent. solution of malachite green in spirit was used as a dressing to the upper half of the inner wound, the lower half being left as a control. Measurements made by tracing the area of the wound through a sheet of clear cellulloid showed that the portion of the wound sprayed with the malachite green developed epithelium much more rapidly than the control portion ; the average increase of the epithelial edge in one week being 0·59 centimetres for the malachite green treated portion and 0·38 centimetres for the control portion.

He was seen some months later walking almost perfectly in a special boot to prevent foot-drop.

Bacteriological Notes.

On admission. *B. sporogenes*, *B. tertius*, *Streptococcus pyogenes*, *B. proteus*, *Staphylococci* (a few). Diphtheroids. In films : Tetrads also seen.

July 25th. *B. sporogenes*, *B. tertius*, *Streptococcus pyogenes*, *Staphylococcus* (a few). Diphtheroids.

July 31st. No anaerobes. *Streptococcus pyogenes* (only).

August 21st. *Streptococcus pyogenes* (only).

September 1st. *Streptococcus pyogenes*, most abundant. A few *B. pyocyaneus* and *B. proteus*.

Case No. 3. Wounded (bullet) 7.7.16. Admitted 21.7.16. Discharged 26.10.16.

History. At the Base a considerable portion of the right quadriceps was removed for local gas gangrene.

Condition on admission (14th day). His injuries were : (1) an almost healed bullet wound of left forearm, with a fracture of the ulna 1½ inches above the wrist. This wound showed no evidence of sepsis and was almost healed. (2) On the anterior aspect of the right thigh there was a large wound extending over its whole surface deeply into the muscles. Much muscle had been removed for gas gangrene. (3) On the posterior surface of the left thigh was a more superficial wound, the skin and deep fascia only being involved. These two latter wounds had fairly clean granulating surfaces. General condition good ; no fever.

Treatment. The left forearm was put up in plaster and gave no further trouble, the bone uniting in good position and no limitation of movement resulting. On August 6th the wound of the right thigh was sutured, but the tension on the stitches was very great owing to the amount of scar tissue that had been formed in the depths of the wound, and, in consequence, the stitches had to be removed early, limiting the success of the operation. Healing progressed slowly but without any set-backs.

Bacteriological Notes.

On admission. No anaerobes were found. *Staphylococcus*, diphtheroids, *B. proteus*, *Streptococci* (a few), *M. tetragenus*, and a coliform bacillus.

July 28th. *B. proteus* and *Staphylococcus*.

August 19th. *Staphylococcus* and a coliform fermenting glucose with production of acid but having no effect on lactose, dulcite, and mannite. As regards cane-sugar a slight amount of acid was produced in the first 24 hours, but the broth shortly after again became alkaline.

August 21st. Very few microbes seen in films. *Staphylococcus* and diphtheroids isolated.

September 18th. Right thigh : *Staphylococcus* and diphtheroids. Left thigh : *Staphylococcus*, diphtheroids, and a few *Streptococcus equinus*.

Note. This case is again remarkable for the absence of *Streptococcus pyogenes* throughout the whole period of observation ; the progress of the case was quite uneventful.

Case No. 4. Wounded (shell) 1.7.16. Admitted 25.7.16. Discharged 18.12.16.

Condition on admission (24th day). The patient was very pale and debilitated, but without fever.

Wounds. There was a large, unhealthy looking, granulating surface extending from the point of the shoulder 4 or 5 inches down the upper arm. This was continuous with a granulating surface involving the axilla and anterior axillary fold. There was a separate wound on the outer border of the scapula which communicated with the axillary wound. X-rays showed a large number of minute pieces of metal in axillary region, arm, and side of chest. The humerus was fissured, but there was no displacement. There was extensive bruising of the nerves of the brachial plexus, giving rise to severe neuralgic pain and partial paralysis of the arm, forearm, and hand.

Treatment. An attempt was made to reduce the size of the wound on August 3rd by suturing, without much success. Treatment largely consisted of moist saline dressings. The progress was slow but satisfactory. He was discharged, almost completely healed, on December 18th, the nerve lesions being still troublesome but improving.

Bacteriological Notes.

On admission. *B. sporogenes*, a tetanus-like organism, *B. sphenoides*, *Streptococci*, *Staphylococci*, *B. proteus*, diphtheroids, and *M. tetragenus*.

August 19th. *B. proteus* and *B. pyocyaneus*, very numerous.

August 24th. *B. proteus*, numerous, and others.

September 18th. *B. proteus*, *B. pyocyaneus*, and a coliform bacillus, which produced acid in glucose broth but had no action on lactose, saccharose, dulcite, or mannite.

Remarks. The outstanding feature of this case was the unhealthy aspect of the granulations ; they were pale, soft, and bled easily. The most constant organism present was *B. proteus*.

Case No. 5. Wounded (shrapnel) 7.7.16. Admitted 26.7.16. Discharged 17.12.16.

History. At the Base he was operated on for secondary haemorrhage, the left common interosseous artery being tied. No history of gas gangrene.

Condition on admission (19th day). On the right upper arm there was a clean granulating surface about 3 inches long on the inner side, not extending below the deep fascia. On the right side of the chest, a little below the clavicle, was another clean granulating surface, no injury of the deeper tissues being found.

There was a through and through wound of the left forearm with a compound fracture of the ulna. Both entry and exit wounds were discharging freely. The right median nerve had been bruised, and there was severe neuralgic pain in the palm of the hand and much wasting of the hand muscles, especially the interossei.

Treatment. The fracture was put up in plaster with windows for dressings as soon as the discharge lessened under wet saline dressings. Progress was uneventful, there being no fever throughout the progress of the case ; the right median nerve was cut down on by Mr. Low, after the wound over it had healed soundly. It was found involved in scar tissue, from this it was freed and a piece of fascia stitched over it. This only relieved the pain for a time, and the patient was still suffering considerably when he left the hospital. The fractured ulna united soundly in good position.

Bacteriological Notes.

On admission. Pus from the left forearm showed many microbes, Gram-negative bacilli, *Streptococci*, diphtheroids, and Gram-positive bacilli. Cultures from forearm gave a few *B. sporogenes* and *B. tertius*, many *Streptococcus pyogenes*, and a coliform bacillus.

Some of the superficial wounds gave cultures of *Streptococcus pyogenes*, *B. proteus*, and a diphtheroid.

August 4th. Chest wound gave *Staphylococcus* only.

August 13th. Chest wound gave *Staphylococcus* only.

August 21st. The wounds connected with the fracture of the left ulna showed large numbers of *Streptococcus pyogenes* only.

Case No. 6. Wounded (bullet and shrapnel) about 18.7.16. Admitted 29.7.16. Discharged 17.11.16.

History. The patient lay out for 3 days after being wounded. Amputation of the left leg in the middle of the thigh was performed at the casualty clearing station.

Condition on admission. 1. Small scalp wound: suppurating but healing. 2. Left hand, wrist, &c.: many small wounds cleaning and healing. 3. Right forearm: large flesh wound, exposing the tendons; this wound was cleaning satisfactorily. 4. Right upper arm: large flesh wound exposing the muscles; this wound was granulating satisfactorily. 5. Right foot: a perforating bullet wound which had caused a comminuted fracture of the third meta-tarsal, rather septic. 6. Amputation stump: the bone was much exposed, the marrow fungating, and the muscles and skin tending to fall away; there was much suppuration but no extensive sloughs.

General condition was very fair, not much fever (maximum 100° F.). He complained greatly of indigestion.

Treatment. Wounds dressed with 5 per cent. saline until clean, afterwards with normal saline. Extension applied to the soft parts of the stump. Progress was very rapid and without any set-backs. When the stump was nearly healed a secondary amputation was performed, the wound being loosely closed and dressed from the beginning with wet dressings of 5 per cent. saline. There was very little constitutional disturbance and healing was very rapid, a good stump remaining. Some sequestra were also removed from the right foot.

Bacteriological Notes.

On admission. Films from stump showed diplococci and a long *Streptococcus*, coliform bacilli, and a Gram-positive bacillus. No anaerobes could be isolated from this situation. *Streptococcus*, mostly of the *faecalis* type, but *pyogenes* also present. Films from the foot showed *Streptococcus* (long chained) and *B. sporogenes.*

August 25th. Cultures from the foot showed *B. proteus*, diphtheroid bacilli, *Streptococcus pyogenes*, and *B. sporogenes.*

October 19th. Three days after secondary amputation practically pure culture of *Streptococcus pyogenes* with a few diphtheroids.

Case No. 7. Wounded (bullet) 9.7.16. Admitted 29.7.16. Discharged 28.12.16.

History. Patient was operated on at the Casualty Clearing Station for sepsis.

Condition on admission (20th day). General condition good.

Wounds. The entry wound was near the acromion on the right side, which communicated by a sinus about 4 inches long with a wound near the inferior angle of the scapula; there were two other wounds lower down on the back, in the right side of the upper lumbar region, which were evidently the result of incisions made for drainage. The scapula was fractured, X-rays showing comminution of the axillary border. A fair amount of rather thin pus.

Treatment. Wet saline dressing. Progress was rather slow but uneventful. On October 18th the remaining wound at the inferior angle of the scapula was explored, some detached pieces of bone removed and drainage improved.

Bacteriological Notes.

On admission. Films showed nothing but *Streptococcus.* Cultures showed large numbers of *Streptococcus pyogenes* and an occasional *Staphylococcus.* No anaerobes.

August 4th. Pure *Streptococcus pyogenes.*

August 22nd. *Streptococcus pyogenes* and *Staphylococcus.*

October 2nd. *Streptococcus pyogenes* and *Staphylococcus* in about equal numbers.

Case No. 8. Wounded (shrapnel) 19.7.16. Admitted 30.7.16. Discharged 12.12.16.

History. At the Base extensive incisions were made for gas gangrene.

Condition on admission (11th day). General condition was fairly good, and there was no great degree of fever.

Wounds. Over the right parietal bone there was a scalp wound about 1½ inches in length. This extended down to the bone, which was bare of periosteum, and the outer table of the skull was fractured. No cerebral symptoms were present.

There was a compound fracture of the proximal phalanx of the little finger of the left hand. The wound was granulating but some bare bone could be felt.

The principal wound was situated in the left thigh; originally the projectile entered somewhere in the region of the anterior superior iliac spine and passed out a few inches lower down the thigh, and the wounds about to be described were almost entirely the result of incisions made to combat the gas gangrene.

The largest wound extended from the anterior superior iliac spine down the front of the thigh to just above the knee, being about 5 inches broad in the upper part of the thigh. In the upper and outer region of the thigh wound there was a considerable loss of tissue.

There were several smaller wounds, linear incisions situated over the calf and the posterior aspect of the thigh, and, lastly, there was a linear wound about 4 to 5 inches long in the abdominal wall over the outer part of the left iliac fossa.

Most of these wounds were very clean and there was no pocketing or sloughing; and the smaller ones were healing rapidly.

Treatment. Wet saline dressings. It was in this case that perforated celluloid was first used to prevent the dressings from sticking, a device introduced on account of the excessive pain caused by the removal of gauze which stuck to a raw surface. The result was most satisfactory.

Progress. On the whole, it was very rapid and satisfactory; the only event worth recording in the earlier stages of the case was an attack of erysipelas, which commenced at the upper part of the wound, which had been dressed with ointment, and slowly spread down the leg. It was of a very mild type.

The wounds were later successfully skin-grafted, and when the patient was discharged on December 12th there remained only a sinus in the scalp leading down to necrosed bone.

Bacteriological Notes.

On admission. Films showed enormous numbers of bacteria, coliform bacilli, *Streptococcus*, tetrads, and sporing bacilli. Cultures: *B. sporogenes*, *B. tertius*, *B. proteus*, and *Streptococcus pyogenes*, and an unnamed anaerobic bacillus.

August 21st. Culture made from various situations showed *Streptococcus pyogenes* in the upper part of large wound. Diphtheroids only in the lower unhealthy portion.

August 22nd. Culture shows pure *Streptococcus pyogenes.*

August 24th. *Streptococcus pyogenes* and *B. pyocyaneus.*

August 30th. *Streptococcus pyogenes* and *Staphylococcus.*

September 5th. Chiefly *Streptococcus pyogenes* and a few *Staphylococcus.*

November 1st. *Streptococcus pyogenes* and a few *Staphylococcus.*

Case No. 9. Wounded (bullet) 19.7.16. Admitted 30.7.16. Discharged 6.1.17.

History. Gas gangrene developed in right thigh, which was treated by large incisions.

Condition on admission (11th day). General condition was not very good, the patient's colour being earthy and his pulse rather feeble.

Wounds. There was a bullet wound of the right elbow region, the entry wound being over the head of the radius and the exit one over the internal condyle with much comminution of the bones and suppuration of joint and both wounds, but no surrounding inflammation. X-rays showed fracture of the humerus, ulna, and (most probably) the radius, just below the head.

The right thigh had a single very extensive wound on the outer and posterior aspect, extending from the great trochanter to the lower border of the popliteal space. This wound extended deep into the intermuscular planes. It was about 5 inches broad at its widest part, and showed a great tendency to sag open. The surface was clean and the suppuration moderate.

Treatment. Elbow splinted on an angular splint and saline dressings applied both to thigh and elbow region.

Four days after admission the thigh wound was brought together by deep and superficial stitches, large drainage tubes being inserted into the deep muscular plains. This considerably lessened the area of the wound in the central portion of the thigh and, although all the stitches did not hold, the general result of the operation was very satisfactory. Healing was complete by the middle of October.

At first the wounded elbow-joint remained very quiescent, but later drainage became unsatisfactory and the inflammatory condition became more acute. Incisions were made into the joint, but drainage remained unsatisfactory and the

patient's general condition became daily worse. On September 3rd the arm was amputated above the elbow. After this the patient's general condition rapidly improved and the stump healed satisfactorily, except that a small ring of necrosis of the distal end of the humerus caused a sinus to persist until December. On the removal of this necrosed bone the sinus rapidly closed.

The patient was discharged on January 6, 1917, with perfect movements of the leg, and the right arm had a sound stump.

Bacteriological Notes.

On admission. *B. sphenoides*, *Streptococcus pyogenes*, diphtheroids from the thigh, *B. proteus*, and *Streptococcus pyogenes* from the elbow.

August 3rd. *B. proteus* and a coliform bacillus giving fermentation reaction of *B. coli communis* and *Streptococcus pyogenes* from the elbow.

Thigh : *B. sphenoides*, *B. proteus*, *Staphylococcus*, and *Streptococcus pyogenes*.

August 11th. *Streptococcus pyogenes* and a few *Staphylococcus* from thigh.

August 21st. From elbow many *Streptococcus pyogenes*, a few *Staphylococcus* and *B. pyocyaneus*.

September 4th. Only *Staphylococcus* and *B. pyocyaneus*.

October 1st. Thigh : diphtheroids and *B. pyocyaneus*. Arm : *Staphylococcus* and *Streptococcus*.

October 16th. Wound of thigh almost healed, anaerobic cultures. *Streptococcus pyogenes* and a few tetrads.

Case No. 10. Wounded (bullet) 23.7.16. Admitted 31.7.16. Discharged 25.9.16.

History. Five days after the injury he was operated on for cellulitis, an incision being made in the front of the thigh, from which the bullet was extracted and pus evacuated.

Condition on admission. Patient had a superficial wound about 3 inches long situated on the right buttock close to the anus. This wound was cleaning well. Opposite this was a penetrating wound of the left gluteal region, the entry wound being quite close to the anus and admitting a probe for about 4 inches deep to the gluteal muscles ; there was a scanty discharge of blood-stained pus. In the front of the thigh situated near the anterior border of the tensor vaginae femoris was an incised wound ; from this wound a probe could be passed for 2 or 3 inches deep to the muscles in the direction of the wound of entry. The left buttock was swollen, but there was no extensive inflammatory induration. A considerable amount of deep haemorrhage had apparently taken place, the skin over the front and outer aspect of the thigh being much discoloured by effused blood. X-rays showed no injury to the bone, and no foreign body could be detected.

Progress. After the first ten days the progress of the wound was quite uneventful. The patient was discharged on September 25th, 1916, with all the wounds healed and but little temporary limitation of the movements of the hip-joint.

Bacteriological Notes.

On admission. Films showed many streptococcus, some Gram-negative bacilli, and a very few bacilli resembling *B. welchii*. Cultures gave a few colonies of *B. welchii*, many of *Streptococcus* and *B. proteus*.

August 8th. *Streptococcus pyogenes* and *B. proteus*.

August 24th. *Streptococcus pyogenes*, diphtheroid and a few *B. pyocyaneus*.

August 29th. A few colonies of *Staphylococcus* and *Streptococcus pyogenes*.

Case No. 11. Wounded (bullet) 24.7.16. Admitted 31.7.16. Discharged 5.10.16.

Condition on admission. General condition good. The patient had a through and through wound in the right upper arm, midway between the axilla and the elbow ; and a drainage tube had been passed right through beneath the biceps. The wound situated on the inner aspect of the arm was considerably the larger and more lacerated. Both wounds were fairly clean, but a quantity of thin blood-stained discharge was escaping. X-rays showed the humerus to be uninjured and that no foreign bodies were present.

Treatment. At first 5 per cent. saline packs, later normal salt was used. Healing was uneventful.

Bacteriological Notes.

On admission. Films only showed streptococci. Cultures in addition to *Streptococcus pyogenes* proved the presence of *B. welchii*.

August 1st. *Streptococcus pyogenes* together with a few *B. pyocyaneus* and a coliform organism.

August 28th–29th. *Streptococcus pyogenes.*

September 18th. *Streptococcus pyogenes* and also a few colonies of *B. welchii.*

Case No. 12. Wounded (shell) 29.7.16. Admitted 3.8.16. Discharged 27.9.16.

History. Operated on for gas gangrene at Boulogne two days after the injury.

Condition on admission. The wound was situated on the right deltoid region, being roughly oblong in shape, about 7 inches long and 5 inches broad. There was much loss of tissue, the humerus being exposed but quite uninjured in the centre of the wound. The base of the wound was very dirty, covered in many places with sloughs, and the skin edges were very irregular, a large flap hanging down posteriorly. The dressings were excessively painful.

Treatment. Five per cent. saline packs changed 2-hourly, the wound surface being protected by a sheet of perforated celluloid which entirely eliminated the pain of the dressings.

In two days under this treatment practically all the sloughs had disappeared and the surface looked like clean raw meat. Then the posterior flap was brought over the wound and fixed in position with strapping. With simple normal saline dressings the granulations became oedematous, but when pressure was applied with a firm bandage they took on a healthy appearance. The subsequent progress was uneventful, and perfect healing resulted. There was no limitation of the movements of the arm.

Bacteriological Notes.

On admission. Films showed enormous numbers of bacteria, principally diplococci, large Gram-positive and Gram-negative bacilli, and coliform bacilli. Cultures, *B. sporogenes*, *B. tertius*, *Streptococcus pyogenes*, *Streptococcus salivarius*, coliform bacilli, *Staphylococcus.* A bacillus exactly like *B. tetani* was also seen in a trypsin-blood-broth culture, but was not isolated.

August 17th. Films showed only cocci and diphtheroids. Cultures, *Staphylococcus albus* and *aureus* and *Streptococci.*

August 19th. *Streptococcus pyogenes* and *B. pyocyaneus.*

August 29th. *Streptococcus pyogenes.*

September 1st. *Streptococcus pyogenes* and a few *Staphylococcus.*

Case No. 13. Wounded (shell) 30.7.16. Admitted 3.8.16. Discharged 28.12.16.

Condition on admission (4th day). There was a large sloughing wound with irregular ragged edges, corresponding roughly in size to the area of the right scapula, over which it was situated. The supra and infra spinatus muscles were in large part destroyed. The scapula was extensively fractured, most of the spine being broken away, and there was a considerable number of loose pieces only partially covered with periosteum. No fracture of the underlying ribs could be detected, and the right humerus and clavicle were both sound.

General condition was by no means good, the patient looked very thin and hectic, there was a considerable degree of continued fever, the evening temperature usually being 102·5° F. with a daily morning remission of about 2 degrees. There was bronchitis with constant coughing and a good deal of muco-purulent sputum. Examination of the chest, however, did not suggest any intra-thoracic injury.

Treatment. The wound was dressed with 5 per cent. saline packs, the wound surface being protected by a sheet of perforated celluloid. The bronchitis was treated with a vaccine made from *B. influenzae* and *Pneumococcus*, both of which micro-organisms were present in the sputum in enormous numbers.

A blood culture having given a growth of an unclassified *Streptococcus*, in one tube out of ten, a vaccine was prepared and administered, seemingly without the slightest effect. Small doses of a mixed *Streptococcus* vaccine were also given.

Progress. In spite of the continued fever the wound cleaned rapidly, but healing was slow, neither contraction of the surface nor epithelial growth at the edges being at all marked. Several pieces of scapula were removed as sequestra. The surface of the wound was not very good, the granulations being very smooth

and shiny and the discharge scanty and thin, a dusky red blush extending from the edges. Although the bronchitic condition improved, the fever became rather higher and more intermittent at the end of the first fortnight.

August 29th. *B. pyocyaneus* having been found abundantly in the wound and in almost pure culture, a small dose (2 million) of a *B. pyocyaneus* vaccine was given. The temperature at once dropped and in a few days became normal, remaining about the normal line for the remainder of the patient's stay in hospital. The condition of the wound rapidly improved.

October 18th. For some time past the healing process had been very slow, portions of the wound often being covered by a membrane and depressions with smooth shiny indolent-looking bases appearing on the granulating surface. Cultures showed the presence of an unclassified *Streptococcus* (see bacteriological notes); a vaccine having been made from this coccus small doses (3 to 5 million) were administered, a very marked improvement occurring as a result of this treatment.

November 25th. The wound had a very healthy bacterial free surface, but contracture and formation of epithelium were both very slow. The unhealed portion of the wound was, therefore, skin-grafted by Steele's method (see Douglas, Fleming, Colebrook, *Lancet*, 1917, ii. 5). The result was most satisfactory, the whole surface being rapidly covered with epithelium, but the scar is very adherent to the underlying bone.

December 28th. The patient was discharged.

In April 1917 the patient was again seen—the ultimate result was very satisfactory, movements of the arm and shoulder being almost perfect. The scar was quite sound, except at one small point where several small pieces of dead bone had worked out. The skin grafts were still quite visible, appearing as white islands in the surrounding reddish brown scar, which was remarkably soft.

Bacteriological Notes.

On admission. Films showed enormous numbers of bacteria, chiefly diplococci, clumps of Gram-positive cocci, tetrads, and large Gram-positive bacilli resembling *B. welchii*, also some free spores. Cultures gave two varieties of *Streptococcus*, one the mannite fermenting type of *pyogenes*, the other an unclassified type resembling *salivarius* but fermenting salicin; *Staphylococci*, Gram-negative streptobacilli, and coliform bacilli. Anaerobic cultures showed *B. welchii* and *B. sporogenes* to be present. A blood culture was positive—a streptococcus with the following characteristics being found in one tube out of the ten into which the blood was distributed. Individual cocci usually round, but some irregular in shape; chains, brevis type; broth culture showed even turbidity; saccharose broth made acid, lactose, raffinose, mannite, salicin, and milk unaffected. Neutral red broth, anaerobic culture gave fluorescence. Sputum: large numbers of *Pneumococci* and *B. influenzae* both isolated.

August 21st. Film showed very few organisms, chiefly *Streptococcus* and a few diphtheroids. Cultures gave *B. sporogenes*, *Streptococcus pyogenes* (mannite fermenting type), *Staphylococcus*, and a Gram-negative bacillus.

August 25th. Blood culture negative.

August 26th. From a deep pocket near the axilla a nearly pure and abundant culture of *B. pyocyaneus* was obtained; the more superficial portion of the wound giving only a few *Staphylococci* and diphtheroids.

August 30th. *Deep pocket*. *B. pyocyaneus* and *Staphylococcus*, no *Streptococcus* isolated; the cleaner portion showing only three colonies of *Staphylococcus*.

September 13th. *Deep pocket*. *B. pyocyaneus* and a few coliform organisms.

September 18th. From the deep pocket anaerobic cultures gave no strict anaerobes, but only *B. pyocyaneus*, *Streptococcus*, diphtheroids, and a few coliform bacilli which produced acid from glucose and slightly from saccharose but had no effect on lactose, dulcite, or mannite. The streptococci isolated on this date were an unclassified type having the following characteristics: individual cocci, usually round but oval forms not uncommon; chains, brevis type; broth culture, a granular deposit was formed leaving the broth clear. Fermentation reactions. Acid produced in lactose and salicin broths; saccharose, raffinose, and mannite unaffected; acid and clot was produced in milk cultures; neutral red broth culture incubated anaerobically gave no fluorescence.

October 4th and 5th. Films made from membranous portion showed only

Streptococcus; anaerobic cultures gave streptococci of the same type as were isolated on September 18th, and one or two colonies of *Staphylococcus*.

Culture (aerobic) from the pocket gave pure *Staphylococcus aureus*. From the membrane anaerobic cultures gave *Streptococcus* of the same type isolated on September 18th, a few tetrads, diphtheroids, and *Staphylococcus*. November 9th, before grafting, only three or four colonies of diphtheroid bacilli.

Case No. 14. Wounded 19.7.16. Admitted 3.8.16. Discharged 27.9.16.

Condition on admission (14th day). General condition good; temperature practically normal. Two wounds were present: (1) the smaller consisted of a superficial wound about 4 inches long by 2 inches broad, situated over the right costal margin; (2) the larger and more severe was situated over the left scapula. In shape and size it was roughly oblong, being about 10 inches in length and the maximum width being about 6 inches. The scapula was extensively fractured but was covered by a clean granulating surface. The lower edge of the wound tended to sag badly.

Treatment. At first 5 per cent. saline packs were applied; later normal salt was used. The edges of the wound were brought together with strapping. Progress was rapid and practically uneventful. The wound over the scapula healed rapidly, and the bone united perfectly.

Bacteriological Notes.

On admission. Films from the wound over right costal margin showed only *Streptococci* and diphtheroids. Cultures gave *Streptococcus pyogenes* and a few *Staphylococcus*. Films from the wound over the left scapula showed only *Streptococci* and diphtheroids. Cultures gave a few colonies of *B. sporogenes* and many of *Streptococcus pyogenes* and *Staphylococcus*.

August 12th. Film from the larger wound, only a few *Streptococci* seen.

August 17th. Cultures from the larger wound, *Streptococcus pyogenes* and *Staphylococcus*.

From the front wound—*Streptococcus pyogenes* only.

August 19th. Cultures from wound of back, *Streptococcus pyogenes* and a few *B. pyocyaneus*.

August 28th. Cultures from wound of back, *Streptococcus pyogenes*.

August 30th. Cultures from wound of back: (i) from an unhealthy looking portion of surface, many *Streptococcus pyogenes*. (ii) From a healthy looking portion of the surface, a few colonies of *Streptococcus pyogenes*.

September 18th. Wound of back. Plate culture gave only one colony of *Streptococcus pyogenes*. Trypsin-blood-broth, *Staphylococcus*, *Streptococcus*, and diphtheroids. Heated pus, sterile.

Case No. 15. Wounded (hand grenade) 2.7.19. Admitted 3.8.16. Discharged 26.9.16.

History. The right leg was amputated below the knee at the Casualty Clearing Station, the wound being sewn up. The left leg was amputated about 4 inches above the knee for sepsis at the Base, the guillotine method being employed.

Condition on admission (32nd day). The right stump 4 inches below the knee joint had healed. The left stump showed a circular wound about 4 inches in diameter which was granulating well, the bone being just covered. Just above the right elbow there was a marked deformity which X-rays showed to be due to a fracture of the external condyle of the humerus; there was no note as to how this had been treated, but union was firm.

Progress was quite uneventful, and when the patient was discharged the left stump was quite healed except for a minute spot.

Bacteriological Notes.

On admission. All the cultures taken were overgrown with *B. proteus*.

August 17th. Cultures gave pure *Streptococcus pyogenes*.

August 31st. *Streptococcus pyogenes* and *Staphylococcus*.

September 13th. *Staphylococcus* and diphtheroids in about equal numbers.

Case No. 16. Wounded (shell) 19.7.16. Admitted 3.8.16. Discharged 12.12.16.

History. Patient arrived at the Base on July 21st, where he remained until August 3rd, suffering from a left haemothorax in addition to the wounds.

Condition on admission (15th day). The patient's general condition was

good; shortness of breath was the only symptom of the haemothorax. The chest signs were very slight, some impairment of resonance and of the breath sounds on the left side being all that could be made out. His wounds were both situated high up on the shoulders, on the right side there was a deepish track leading towards the clavicle from the supra-spinous fossa. On the left side the wound, which was in a similar situation, was apparently more superficial. X-rays showed the shadow to be increased over the whole of the left side, but the diaphragm moved normally and the dome was not depressed. The heart was somewhat pushed over to the right side. There were two or three small pieces of metal embedded in the right shoulder.

Progress was rather slow, owing to the presence of the foreign bodies, three of which were removed and drainage improved by counter openings. The haemothorax gave no further trouble and was seemingly completely absorbed.

Bacteriological Notes.

On admission. Culture gave *Streptococcus pyogenes* and *Staphylococcus*, also some very minute colonies of a thin curved bacillus, which failed to grow in subculture. Films also showed diphtheroids.

August 22nd. Cultures gave a few colonies of *Staphylococcus* and one colony of *Streptococcus*.

September 5th. Left shoulder almost healed—*Staphylococcus* only. Right shoulder—*Streptococcus pyogenes* in pure culture.

October 16th. Right shoulder—many *Streptococcus pyogenes* and a few *Staphylococcus*.

Case No. 17. Wounded 12.7.16. Admitted 9.8.16. Discharged 4.9.16.

History. At the Base two or three days after being wounded, he had secondary haemorrhage on two occasions and a severe attack of erysipelas which was treated with streptococcus vaccine.

Condition on admission (28th day). The wound of entry, in front of the lobe of the right ear, quite small and healed; the exit wound behind the sterno-mastoid was larger and more ragged but quite clean. All signs of erysipelas had disappeared.

Progress. Rapid and very satisfactory, the wound healing rapidly from the bottom.

Bacteriological Notes.

On admission. *Streptococcus pyogenes*, diphtheroids and *B. proteus*.
August 18th. *Staphylococcus* only.
August 19th. *Staphylococcus* only.
August 22nd. *Staphylococcus* only.

Case No. 18. Wounded (bullet) 19.7.16. Admitted 21.8.16. Discharged 14.11.16.

History. At the Base on July 22nd, amputation was performed through middle of right thigh (flapless method).

Condition on admission (32nd day). The general condition was good.

Wounds. Amputation stump of the right thigh was clean, but the bone was projecting considerably and the tissues had lost much of the elasticity owing to the formation of scar tissue. There was a small sinus on the posterior internal aspect of the stump about 2 inches long.

Left leg. There was a perforating wound about the middle of the calf, both apertures being small. No injury of the bones or nerves could be found. A moderate discharge of pus was escaping from the wounds.

Progress was almost uneventful, occasionally there were slightly inflamed spots about the stump, but on the whole healing was rapid. A ring of bone separated from the end of the femur about the middle of September but in spite of this the bone still protruded beyond the soft tissues. By this date the left leg had healed. In the middle of October a secondary amputation was performed, about 3 inches of the shaft of the femur being removed through an irregular incision which was almost completely closed by sutures. The stump was dressed from the first with very wet hot 5 per cent. saline packs and under this treatment healing took place rapidly with but little suppuration. The patient was discharged on November 14th, 1916, just four weeks after the secondary amputation, with a soundly healed stump.

Bacteriological Notes.

On admission. Cultures from the left calf gave *Streptococcus pyogenes* and

a heat-resisting *Streptococcus*, while the stump gave *B. pyocyaneus*, diphtheroids, and a Gram-negative bacillus.

September 5th. *Staphylococcus*, *Streptococcus pyogenes*, diphtheroids, and *B. proteus* were grown from the stump.

(This was one of the wounds from which a bacillus apparently identical with *B. diphtheriae* was isolated.)

September 18th. Gave a number of colonies similar to the above.

October 5th. The cultures were overgrown with *B. proteus*.

October 20th. After the secondary amputation *Streptococcus pyogenes* was found in considerable numbers.

Case No. 19. Wounded (shell) 29.7.16. Admitted 21.8.16. Discharged 14.11.16.

History. Gas gangrene developed at the Base and was treated with multiple incisions.

Condition on admission (23rd day). General condition was good. The wounds were situated on the posterior and outer aspects of the thigh, the principal one being on the posterior aspect and extending into the intermuscular planes. In addition to this there were some twenty-five other wounds, evidently incisions for the treatment of gas gangrene, most of them extended through the deep fascia.

Progress was at first rapid and by September 15th all the smaller incisions had healed, but the principal wound showed some tendency to digestion of the epithelial edge and the granulating surface. Local applications of nitrate of silver and brilliant green, however, improved the condition, and the patient was discharged on November 14th, completely healed.

Bacteriological Notes.

On admission. Films from the large wound showed *Streptococci* and *Staphylococci*. Cultures gave colonies of *Streptococcus pyogenes* and *B. pyocyaneus*.

October 5th. Plates overgrown with *B. proteus*.

October 9th. Plates overgrown with *B. proteus*.

October 10th. Diphtheroids only.

When the large wound showed areas of digestion of the surface and epithelial edge, films were made from these situations and compared with films made from portions of the surface where the granulations had a more healthy look. On staining and examining these a very marked difference was found in respect to the number of organisms seen, the films made from the unhealthy looking portion were crowded with streptococci and coliform bacilli (most probably *B. proteus*) while those made from the healthy surface contained practically no micro-organisms.

Case No. 20. Wounded 12.6.16. Admitted 22.8.16. Transferred 23.6.17.

History. Tetanus having developed, the left leg was amputated at the Base through the middle of the thigh by a guillotine operation. The stump remained in a very septic condition until continuous irrigation with hypertonic saline was employed, when considerable improvement was noticed.

Condition on admission (61st day). The left leg stump had almost healed over, but the bone was protruding about 2 inches beyond the soft parts.

Right leg. There was a compound comminuted fracture of the tibia and fibula just below the knee. Over the fracture of the tibia was a deep hole, opening on the inner side of the leg; stretching from this towards the outer side was a granulating surface surrounded by a considerable area of scar which was hard, inelastic, and very adherent to the deeper tissues. This scar extended over the region of the neck of the fibula to almost behind the knee. The fracture showed very little evidence of union. The external popliteal nerve had been divided just above the head of the fibula. The patient's general condition was poor. There was a large splint sore on the heel. X-ray showed that the whole of the tibia had been rarefied by osteomyelitis.

Progress. At first it was very slow and unsatisfactory, as after healing considerably the scar would break down; the hole over the head of the tibia showed no signs of healing. If the patient had not lost the other leg amputation undoubtedly would have been the proper treatment. In the beginning of October the wounds were explored and some counter openings made to improve drainage. After this the patient's general condition began to improve. In November a more extensive operation was performed, several pieces of dead bone being

removed and drainage made as free as possible. Until February 1917 very little of importance took place, the patient's general condition steadily improved, and a radiogram after bismuth injection showed that a considerable amount of new bone had been formed and that sequestra were separating inside the shaft of the tibia.

Towards the end of February a mild attack of erysipelas intervened, and shortly afterwards a new sinus formed about 3 inches above the internal malleolus. Further X-ray photographs pointed to the whole of the original shaft of the tibia having necrosed and formed a sequestrum lying inside the new bone. About the middle of April the tibia was, therefore, opened up, and the sequestrum was removed from the whole length of the shaft through two incisions, one being an enlargement of the original hole, on the inner side of the head and shaft of the tibia, the lower one being above the internal malleolus. After this operation healing was rapid, the lower wound healing very quickly. Improvement continued until the patient was transferred on the closing of the Inoculation Department wards. The condition at that time was as follows: The upper wound over the head of the tibia had almost healed, the epithelium spreading downwards into the large cavity in the head of the bone, but there was a sinus over the head of the fibula leading to a small sequestrum. The patient's general condition was excellent.

Bacteriological Notes.

On admission. Films showed very numerous bacteria amongst which were large capsulated Gram-negative bacilli, *Streptococci* (two varieties), diphtheroids tetrads. Cultures gave two varieties of coliforms, *Streptococcus faecalis* and *Streptococcus pyogenes*, *B. pyocyaneus*, *Staphylococci*, *M. tetragenus*, diphtheroids, *B. tertius*, and *B. sporogenes*.

August 26th. Films showed *Streptococci*, diphtheroids, a coliform bacillus, tetrads, and a long slender Gram-positive bacillus.

August 31st. *B. sporogenes* and *B. tertius*, *Streptococcus pyogenes*, *B. proteus*.

September 18th. *Streptococcus pyogenes*, *Staphylococcus*, and diphtheroids.

November 8th. From sequestra removed at operation, *B. sporogenes*, *B. sphenoides*, and *B. tertius*. *Streptococcus pyogenes* and *B. proteus* were all obtained by culture and in addition to these some tetrads, *Staphylococcus*, and Gram-positive bacteria were seen in films.

November 13th. *Streptococcus*, diphtheroids, *Staphylococcus* coliform bacilli, and *B. proteus*, also *B. sporogenes* and *B. tertius* from the heated pus.

April 13th, 1917. From a sequestrum removed at the second sequestrotomy, *B. sporogenes*, *B. tertius*, *Staphylococcus*, *Streptococcus*, and *B. proteus*.

April 18th. From the pus, *Streptococcus*, *Staphylococcus* and *B. proteus* were isolated.

May 5th. *Streptococcus* and *Staphyloccus*, diphtheroids, and *B. proteus*.

Case No. 21. Wounded (bullet) 12.8.16. Admitted 27.8.16. Discharged 14.11.16.

History. Operated on at Casualty Clearing Station for gas gangrene.

Condition on admission (15th day). General condition good, except for marked nervousness and dislike of noise. The patient had a large ragged wound about 9 by 6 inches in the front of the left thigh, extending deeply into the muscles. The femur was uninjured. This wound had a fairly clean surface, but one or two sloughs were still attached. In addition there were two linear wounds about 5 inches long, the result of incisions on the outer side of the same thigh. On the right thigh there were two small wounds, one about ¼ inch in diameter in front of the great trochanter, apparently almost healed, the other about 1 inch long, situated in the right groin. This wound had a track leading from it directly backwards and from it a little thick pus was oozing, but there was no surrounding inflammation.

Progress was rapid, especially at first, the large wound filling up well, and the smaller ones healing very rapidly. Later the healing became much slower, but by November 14th all the wounds were healed. The movements of the leg were seemingly in no way impaired, the quadriceps muscles acting well and strongly.

Bacteriological Notes.

Films from large wound showed enormous numbers of bacteria. Gram-positive cocci, single cocci, diplococci, and tetrads, diphtheroids, Gram-negative bacilli, and Gram-negative diplococci.

Cultures gave *B. sporogenes*, *B. sphenoides*, *Streptococcus pyogenes*, *Streptococcus equinus*, and an unclassified *Streptococcus*, which produced acid in saccharose and raffinose broths but had no action on lactose, mannite, or salicin. Milk was neither acidified nor clotted and neutral red broth (anaerobic) was unaffected. The individual cocci were round and regular and the chain short. *B. proteus*, a coliform bacillus, and diphtheroids were also found.

September 4th. *Streptococcus pyogenes.*

September 6th. *Streptococcus pyogenes.*

October 5th. Cultures showed chiefly *Staphylococcus* and a few *B. pyocyaneus.*

Case No. 22. Wounded (bullet) 13.8.16. Admitted 11.9.16. Discharged 24.6.17.

On admission (29th day). General condition was good, temperature only slightly and intermittently raised. There was a wound about 7 inches long and 4 inches broad over the outer side of the middle of the thigh leading down to the bone, which was fractured in two places.

Treatment. Extension was continued with a modified Thomas splint, the wound being intermittently irrigated with 5 per cent. saline and drainage secured by means of a water suction pump.

Progress. Under this treatment the condition steadily improved, the bone uniting in very fair position, and the wound healing slowly but with the scar somewhat adherent to the bone, and a persistent discharge of pus from a cavity containing sequestra.

In February 1917, and again in June, some of these sequestra were removed by open operation, but probably not with complete success, since after the patient had left the hospital with the wound healed it again broke down.

In view of the fact that such operations are almost invariably followed by increased suppuration associated with an abundant proliferation of all the bacteria in the wound, it was decided to follow up the second operation immediately with applications of flavine on gauze. The course of events was, however, in no way changed for the better, for the bacteria proliferated exactly as they had done before and a slight attack of erysipelas developed in the affected limb a day or two after the operation.

Bacteriological Notes.

On admission. Films showed only a moderate number of bacteria, *Streptococci* and a coliform bacilli being noted. Cultures gave *Streptococcus salivarius*, a Gram-negative coccus, and a coliform bacillus.

September 25th. *Streptococcus salivarius* and *Streptococcus equinus*, also *B. proteus.*

November 8th. *Streptococcus pyogenes* and *Staphylococcus.*

November 23rd. *B. proteus*, *Streptococcus pyogenes*, and diphtheroids.

February 9th. *B. proteus* and *Staphylococcus* only.

February 15th. *B. proteus* and *Staphylococcus* only.

February 28th. (Two days after operation.) Films showed very large numbers of *Streptococci* and diphtheroids.

March 1st. (After operation.) Films again show many *Streptococci*, diphtheroids, and a Gram-negative bacillus. Cultures gave *Streptococcus pyogenes*, *Staphylococcus*, and *B. proteus*; a few *B. sporogenes* were also isolated by anaerobic cultures.

March 9th. Pure cultures of *Streptococcus pyogenes.*

March 27th. Film showed only *Streptococci* and diphtheroids. Cultures gave *B. proteus*, *Staphylococcus*, and *Streptococcus.*

April 27th. *Streptococcus pyogenes.*

May 3rd. Film showed only a few *Streptococci.*

Case No. 23. Wounded (shell) 3.9.16. Admitted 12.9.16. Discharged 28.12.16.

History. On arrival at the Base patient had developed gas gangrene, and this was treated with multiple incisions.

Condition on admission (9th day). There was a wound over the right buttock, midway between the great trochanter and the natal cleft; this had evidently been enlarged by a deep cross-shaped incision and was kept open by a large drainage tube which passed about 3½ inches into the buttock. The wound surface was fairly clean, but there was a considerable amount of blood-stained stinking pus from the depths. In the left buttock, over the gluteus maximus

was a wound from which a sinus extended about 4 inches in the direction of the anus. Over the upper part of the left thigh and buttock there were nine other wounds the result of incisions, each about 3 inches long and, for the most part, only extending through the skin and subcutaneous tissues. One, however, in the neighbourhood of the wound caused by the projectile was deeper, extending well into the muscles. There was also a wound in the natal cleft close to the anus. These wounds were all fairly clean.

The patient's general condition was good but there was some intermittent fever.

Progress. For the first three or four weeks the patient had irregular attacks of fever, due to temporary failures of drainage, but after that period there were no constitutional symptoms. Various treatments were employed to encourage the growth of epithelium, such as a 1 per cent. solution of brilliant green, red lotion, &c., and in the end the wound healed very satisfactorily.

The patient was discharged on December 28th.

Bacteriological Notes.

On admission. Films showed *Streptococci*, diphtheroids, coliform bacilli, and spore-bearing bacilli. Cultures gave *B. sporogenes*, *Streptococcus pyogenes*, *B. proteus*, a coliform bacillus, and diphtheroids.

October 4th. Films showed many *Streptococci*. Cultures, practically pure *Streptococcus pyogenes*.

October 31st. Cultures gave *Streptococcus pyogenes*, diphtheroids, and *Staphylococcus*.

Case No. 24. Wounded (shell) 27.8.16. Admitted 15.9.16. Discharged 24.1.17.

On admission (18th day). General condition was not very good, the patient being rather worn out with pain, sepsis, and haemorrhage. There was a large, deep, ragged wound, midway between the point of the right shoulder and the root of the neck, roughly measuring 5 by 4 inches. The clavicle and scapula were both extensively fractured and several pieces of bone were present in the wound, the surrounding muscles being much lacerated. The wound was granulating and showed little suppuration.

Progress. During the first fortnight there was a considerable amount of fever and a dusky red blush spread from the wound down the back to as low as the iliac crests. This blush had not the appearance of erysipelas and soon subsided, after which the wound rapidly filled up and healed. Several pieces of bone were removed from time to time, the clavicle united in fairly good position.

Bacteriological Notes.

On admission. Films showed many *Streptococci*, tetrads or *Staphylococci*, a coliform bacillus, and a few Gram-positive bacilli. Cultures gave *Streptococcus pyogenes*, *Staphylococcus*, a coliform bacillus, and an anaerobic bacillus (? *Vibrion septique*).

September 20th. Film showed *Streptococci* only.

September 21st. Film showed diplococci and some diphtheroids. Cultures gave colonies of *Staphylococcus*, *Streptococcus pyogenes*, diphtheroids, and a coliform bacillus which produced acid and gas in mannite broth but had no effect on glucose, lactose, saccharose, or dulcite.

November 8th. Film showed coliform bacilli, *Streptococci*, and diphtheroids. Cultures gave a coliform bacillus, *Streptococcus pyogenes*, and diphtheroids.

Cultures from heated pus were sterile.

Case No. 25. Wounded (shell) 9.9.16. Admitted 16.9.16. Discharged 28.12.16.

On admission (7th day). The patient had a flesh wound on the outer side of the lower half of the left thigh, in the upper part of which the muscle had herniated through the fascia lata. The surface of the wound was fairly clean, but there were a few sloughs still adhering.

Progress. Under treatment the wound rapidly cleaned and was diminished in size considerably by strapping. Later as healing became very slow the wound was skin-grafted by Steele's method. This at first was very successful, all the grafts adhering, but later the epithelium failed to spread as well as was expected. The patient was discharged on December 28th, the wound then being soundly healed.

Bacteriological Notes.

On admission. Films showed only diphtheroids and cocci. Cultures gave two varieties of diphtheroids, *Staphylococcus*, a few *Streptococci*, and a few Gram-negative bacilli. Anaerobic cultures from heated pus gave *B. sporogenes.*

October 18th. *Staphylococci* and diphtheroids were isolated from the patient's urine.

October 31st. Some days after skin grafting only a few *Staphylococci* and diphtheroids were found in cultures taken from the wound.

Case No. 26. Wounded (bullet) 1.9.16. Admitted 17.9.16. Discharged 28.1.17.

History. Gas gangrene developed at Casualty Clearing Station, which was treated by excision of the gangrenous muscle.

Condition on admission (16th day). There was a large wound over the front and inner side of the right thigh commencing about the level of the apex of Scarpa's triangle and measuring 7 by 4 inches. Just to the outer side of this wound was an operation wound. Over its whole area the large wound extended deep into muscles which had been freely excised, the wound surface was covered with fine clean granulations. The patient's general condition was very good.

Progress. The wound healed steadily and satisfactorily, the edges being approximated by strapping. There was, however, some fever for the first fortnight or three weeks. He was much troubled by attacks of severe pain, commencing about the level of the knee and passing down the inner side of the leg: these became more and more frequent and were found to follow the distribution of the internal saphenous nerve. Eucaine solution injected over the course of this nerve in the wound temporarily stopped the pain. On November 27th the internal saphenous nerve was cut and the attacks of pain ceased and did not recur.

On December 20th, healing having become rather slow, the remaining area was covered in with Steele's grafts, the pieces of skin being taken from the anaesthetic area which resulted from the division of the internal saphenous nerve. These grafts took very satisfactorily. Later the thigh was massaged daily as a very extensive and hard mass of scar tissue had formed which apparently so diminished the blood supply as to bring the healing process almost to a standstill. This daily massage was quite painless and appeared to have a very satisfactory result, the remaining small areas being rapidly covered with epithelium.

The patient was discharged quite healed and with full movements of the leg on January 28th, 1917.

Bacteriological Notes.

On admission. Films showed many *Streptococci, Staphylococci*, and a Gram-positive bacillus. Cultures gave *Streptococcus pyogenes, Staphylococcus*, and a few coliform bacilli. Cultures made from heated pus were sterile.

October 3rd. Films showed very few bacteria, *Streptococci*, and diphtheroids. Cultures gave *Streptococcus pyogenes*, diphtheroids, *B. pyocyaneus* and a few *Staphylococci.*

October 26th. *B. pyocyaneus* was isolated and was by far the commonest organism present.

December 16th. Cultures taken before skin grafting gave a few colonies of *Streptococcus pyogenes.*

Case No. 27. Wounded (shell) 6.8.16. Admitted 18.9.16. Discharged 29.1.17.

Condition on admission (43rd day). (1) There was a long narrow wound, almost healed, about 10 inches in length, running obliquely across the back. (2) In the lumbar region was a small depressed wound also almost healed from which a piece of shell weighing 2½ ounces had been removed. (3) Just above the right external malleolus was a small round hole leading down to the tibia the diameter of the orifice being about ¾ inch. (4) On the dorsum of the right foot was a small wound, situated between the metatarsal bones of the great and second toes. (5) Lastly there was a wound over the radial side of the left forearm also nearly healed. X-rays demonstrated a fracture of the left radius, grooving of both the tibia and fibula, and foreign bodies in the neighbourhood of the wounds in the right leg and right foot.

Progress was rather slow, some of the scars tending to break down; all the fractures united soundly Patient was discharged on January 28th, 1917,

with all the wounds healed except that over the lower part of the right tibia where there was still a small sinus.

Bacteriological Notes.

On admission. Films showed *Streptococcus* only. Cultures gave *B. proteus*, *B. pyocyaneus*, *Streptococcus pyogenes*, and a heat-resisting *Streptococcus*.

September 26th. *B. proteus*, *Streptococcus pyogenes*, and diphtheroids by culture.

October 5th. Cultures were overgrown with *B. proteus*.

Case No. 28. Wounded (shell) 20.8.16. Admitted 18.9.16. Discharged 27.12.16.

Condition on admission (28th day). Left stump was healing satisfactorily. A wound of the right thigh 3 inches above the knee measured about 5 by 4 inches and involved considerably more than half the depth of the muscles. The surface was clean and the epithelium was beginning to spread from the edges.

Progress. On November 25th the wound of the right thigh was skin-grafted with Steele's grafts and 'section grafts'[1] with a most satisfactory result, the whole surface being almost healed in three weeks and quite sound in four weeks.

Patient was discharged on 27.12.16 all the wounds soundly healed, the stump being fairly good, but the bone was adherent to the scar. The wound of the right thigh was quite soundly healed.

Bacteriological Notes.

On admission. Films showed very few bacteria, *Streptococci*, and *Staphylococci*. Cultures gave *B. proteus* and *Streptococci*.

October 5th. Culture made but they were all overgrown with *B. proteus*.

October 6th. A few colonies only of diphtheroids and cocci.

Case No. 29. Wounded (shell) 16.9.16. Admitted 29.9.16. Discharged 23.4.17.

History. Large masses of the gluteal muscles had been excised at a Base Hospital in France for localized gas infection and later practically the whole of the right sacro-iliac joint was excised.

Condition on admission (13th day). The patient's general condition was good considering the size and situation of the wound. This was situated in the right buttock and was roughly oval in shape, the long axis measuring about 15 inches, the short axis about 8 inches. Practically the whole of the gluteal muscles had been removed and at its centre the wound was very deep, extending down between the sacrum and ilium so that the abdominal contents bulged into the wound when the patient coughed. The great trochanter lay towards the outer and lower part of the wound covered only by granulations. There was also in the centre of the wound a large mass of necrosed ilium, with tracks going deep to it from the wound surface. The hip-joint was unaffected. The patient was much troubled with cough, large quantities of sputum being brought up, which on examination was found to be crowded with *Pneumococci* and *B. influenzae*. There was a bed sore over the left great trochanter.

Progress. For the first six weeks there was a considerable amount of fever, probably due in part to the bronchial condition.

On October 7th a large piece of necrosed bone was removed from the neighbourhood of the sacro-iliac joint, which greatly improved the drainage of the deeper portion of the wound. After this the wound progressed wonderfully quickly, granulations springing up from the bottom and filling up the cavity, the epithelium also spreading in from the edges. When, later, the contraction of the wound and the spread of epithelium became slow, some forty-five Steele's grafts taken from the patient's thigh were applied to the granulating surface, no special arrangement being adopted. Although several useful centres of epithelial growth were established by this operation, the result was not so good as it would probably have been had the grafts been arranged peripherally and near each other. The advantage of such an arrangement had not, however, been learned at that time.

A second skin-grafting operation two months later, when the formation of scar tissue had become very pronounced, and the granulations in consequence very bloodless, was an almost complete failure. However, under massage to loosen the scar tissue and to promote circulation, healing progressed slowly but surely, and the patient was discharged with the wound healed on April 23rd, 1917.

[1] Douglas, Fleming, and Colebrook, *Lancet*, 1917, ii. 5.

Seen some months later the patient appeared in perfect health, the wound was perfectly sound, the sacro-iliac joint being quite firmly united. Movements of the hip were quite perfect and the patient could walk, run, or jump without any difficulty. The only point noted was that the leg on the injured side was slightly longer than that on the uninjured side. This was corrected by a thick boot sole on the sound side.

Bacteriological Notes.

On admission. Films showed long and short chained *Streptococci*, long and short Gram-negative bacilli, long and short Gram-positive bacilli, tetrads, *Staphylococci*, and diphtheroids. Cultures gave *B. sporogenes* and *B. tertius*, *Streptococcus salivarius* and *Streptococcus equinus*, *M. tetragenus*, diphtheroids, and a coliform bacillus.

October 6th. Films showed *Streptococci* and diphtheroids, there were but few bacteria. Cultures gave *Streptococcus pyogenes*, *Streptococcus salivarius*, and *B. proteus*.

October 7th. Films from the necrosed bone showed diplococci, tetrads, diphtheroids, coliform bacilli, and a large Gram-positive bacillus and a long thin bacillus with an end spore, also many free spores. Cultures gave *B. welchii*, *B. sporogenes* and *B. tertius*, *Streptococcus pyogenes*, and some coliform bacilli. Sputum gave an abundant culture of *Pneumococci* and *B. influenzae*.

November 7th. Film showed only a few *Streptococci* and tetrads.

November 30th. Film showed very few bacteria, one or two cocci and diphtheroids being seen. Cultures gave a few colonies of *Streptococcus pyogenes*, diphtheroids, and *B. proteus*. After this, although many observations were made, the wound was always practically sterile.

It may here be mentioned that cultures taken from the bone that had been removed some months after it had been kept in a dry state gave cultures of *Streptococci*, *B. welchii*, and *B. sporogenes*.[1]

Case No. 30. Wounded (shell) 14.9.16. Admitted 30.9.16. Discharged 27.2.17.

History. After being wounded patient lay out for twenty-four hours. He arrived at the Base on September 16th where he was operated on for gas gangrene.

Condition on admission (16th day). The patient had an extensive wound in the region of the left calf. The left gastrocnemius and soleus had both been almost entirely dissected away, except for the upper one-fourth, together with the skin covering them, on account of extensive gas gangrene ; the long bones were quite uninjured and the wound surface was clean. The foot was in excellent condition.

Progress. At first healing took place rapidly but later it became very slow, the scar becoming adherent to the bones. Later, the treatment adopted was that which has been used for very callous varicose ulcers, layers of gauze being bandaged on from the toes to the knee, each layer having a coat of Unna's paste painted over it. About four layers of gauze and paste were put on and this dressing left for about one week. Under this treatment the epithelium steadily extended, and when the wound had become almost linear a row of Steele's grafts was applied with good results. The ultimate result was that when the patient was discharged on February 27th the wound was scarred over except for a very minute spot. The foot was in excellent position.

Bacteriological Notes.

On admission. Films showed many bacteria, *Streptococci*, Gram-negative bacilli, and diphtheroids. Cultures gave *Streptococcus pyogenes* and *B. pyocyaneus*, and *B. sporogenes*.

October 4th. *Streptococcus*, *B. pyocyaneus*, *Staphylococcus*, diphtheroids, and a coliform bacillus which produced acid in glucose broth but had no effect on lactose, dulcite, or mannite. Saccharose broth was rendered slightly acid after some days.

October 25th. A blood culture, made in consequence of a sudden rise of temperature, was negative.

October 31st. *Streptococci*, diphtheroids and a coliform bacillus by culture.

December 7th. A few *B. pyocyaneus* and diphtheroids.

[1] Cultures were again made from the same piece of bone three years after its removal, with the result that the *B. welchii* and *Streptococcus faecalis* were both isolated.

December 27th. Practically no bacteria seen in films; the cultures, however, gave *B. proteus*.

Case No. 31. Wounded 26.9.16. Admitted 5.10.16. Discharged 6.1.17.

Condition on admission (9th day). The patient had through and through wounds of both thighs. The entry wound of the right thigh had been enlarged by operation, and measured about 4 by 2½ inches. It was situated about the middle of the thigh and had a drainage tube of large size passing horizontally backwards to the exit wound. The left thigh had also two wounds situated about the middle, the entry wound being to the front and inner aspects, the exit wound at the back and outer side; a small drainage tube was inserted into the wound of entry.

The patient was discharged on January 6, 1917, healed and with practically no limitation of movement.

Bacteriological Notes.

On admission. *Right thigh.* Films showed a considerable number of bacteria, diphtheroids, and *Streptococci*. Cultures gave *Streptococcus pyogenes*, *Staphylococcus*, diphtheroids, and coliform bacillus, which produced acid in glucose broth but did not affect lactose, saccharose, dulcite, or mannite. *Left thigh.* Films showed *Streptococci* and diphtheroids. Cultures gave *Streptococcus pyogenes*, *Staphylococcus*, and diphtheroids.

October 18th. *Right thigh.* Cultures gave pure *Streptococcus pyogenes*.

December 5th. *Left thigh.* A small abscess having formed, pure *Streptococcus pyogenes* was isolated from the pus.

Case No. 32. Wounded (shell) 7.8.16. Admitted 7.10.16. Discharged 31.3.17.

History. The left foot was amputated at the C.C.S. a little above the malleoli, antero-posterior flaps being used. He arrived at the Base on August 10th with severe sepsis of the right foot and ankle-joint; the ankle-joint had to be extensively opened up and much bone removed. While at the Base there was extensive septic thrombosis of the external saphenous vein requiring incisions on the outer side of leg.

Condition on admission (61st day). The stump of the left leg was almost healed. The right ankle-joint was extensively damaged, the os calcis and astragalus being much comminuted; there was a large wound on the inner side of the joint from which a tube was passed to the outer side emerging below the external malleolus and rather posterior to the joint. On probing much dead bone could be detected. There was also an incised wound about 2½ inches long on the outer and posterior aspect of the middle of the leg.

Progress. At first there was very gradual improvement in the patient's general condition. On November 23rd the wounds were opened up and much necrosed bone, chiefly pieces of the astragalus and os calcis, were removed, together with much unhealthy granulation tissue; the wound was then packed with gauze to stop the haemorrhage which was free. This packing was removed the next day under gas and the wounds were dressed with 5 per cent. saline packs and 3 per cent. saline baths were employed daily. Under this treatment improvement was very rapid, the suppuration becoming less and less.

Unfortunately a troublesome splint sore developed over the heel. The ultimate result was, however, excellent, the bones uniting solidly with the foot in good position and all the external wounds healing. The patient was discharged on March 31st, 1917.

Bacteriological Notes.

On admission. Cultures from right ankle gave *Streptococcus pyogenes*, *Streptococcus anginosus*, *Staphylococci*, and *B. proteus*. *B. sporogenes* and *B. cochlearis* were isolated by anaerobic methods.

November 7th. Films from right foot showed Gram-negative bacteria, Gram-positive bacteria, and a few *Streptococci*. Cultures gave *B. proteus*, *Streptococci*, and a coliform bacillus which fermented glucose with the production of acid only, saccharose was also slightly acted on, a trace of acid being produced after several days; dulcite, lactose, and mannite were all unaffected. *B. sporogenes* was also found in a culture made from heated pus.

November 8th. *B. proteus*, *Streptococcus faecalis*, and *Streptococcus equinus*.

December 3rd. Cultures overgrown with *B. proteus*.

December 23rd. Almost pure *Streptococcus anginosus*.

January 10th. *B. proteus, Streptococcus pyogenes,* and diphtheroids.
February 11th. *Streptococcus pyogenes.*

Case No. 33. Wounded 28.9.16. Admitted 9.10.16. Discharged 22.1.17.

History. Localized gas infection which developed in the region of the shoulder necessitated excision of much of the left deltoid.

Condition on admission (11th day). The right elbow had a wound over the external condyle of the humerus about the size of a 5-shilling piece; at the bottom of this the head of the radius could be seen, the joint surface being quite exposed. There was a large wound of the right shoulder and upper part of the arm, with much loss of the deltoid muscle, the humerus could be seen at the deepest part of the wound. Most of the skin, &c., had been destroyed, but there was a small flap left in the upper part. The wounds were all very clean.

Progress. Healing took place rapidly, the right elbow joint closing up with little suppuration and no pain. The extensive wound of the right shoulder was slower in healing, several pieces of bone having to be removed from the humerus. When the wound had partially filled in and the granulations were in a satisfactory condition Steele's skin grafts were applied, but considerable difficulty was encountered in satisfactorily dressing the grafted surface and many of the grafts were destroyed in consequence.

The patient was discharged on January 22nd, 1917, all the wounds being soundly healed. The right elbow joint was ankylosed.

Bacteriological Notes.

From the shoulder wound films showed *Streptococci* of two types, oval and round in shape, a coliform bacillus, and large and small Gram-positive bacilli, also diphtheroids and *Staphylococci.* Cultures gave *B. proteus, Streptococcus pyogenes,* and a *Streptococcus* which fermented lactose, saccharose, mannite, and salicin, but did not affect raffinose ; milk was rendered acid and clotted ; neutral red broth cultures incubated anaerobically were rendered florescent, gelatine and coagulated egg were *liquefied.* Diphtheroids and *B. sporogenes.*

From the elbow films showed only very few bacteria, *Streptococci,* coliform bacilli, and diphtheroids being seen. Cultures gave *B. proteus, Streptococcus pyogenes,* and diphtheroids. The heated pus from the elbow gave no growth.

October 11th. Pus from the elbow gave a few colonies of *B. proteus* and *Streptococcus.*

October 13th. A similar result was obtained.

October 17th. Pus from the shoulder. Films showed very few microbes, *Staphylococci,* diphtheroids, and *Streptococci.*

October 18th. Pus from the elbow gave cultures of *Streptococcus pyogenes, Staphylococcus,* and *B. proteus.*

October 27th. Cultures from the shoulder wound gave *B. pyocyaneus* and an unknown anaerobe.

November 8th. Cultures from the elbow gave *Streptococcus* and a few coliform bacilli (no *B. proteus*).

From the shoulder wound *B. proteus* only.

Case No. 34. Wounded (shell) 4.10.16. Admitted 12.10.16. Discharged 27.2.17.

History. Muscle excised at Casualty Clearing Station and again at the Base in France for gas gangrene.

Condition on admission (8th day). There was a large gaping wound on the outer side of the right leg which extended from the knee to the ankle. There was a very extensive loss of skin, fascia, and the muscles of this region, the fibula was fractured, and the outer part of the tibia was exposed in the lower part of the wound for about two inches. In the upper part of the wound was a tube which passed through the wound between the bones, emerging at the back of the calf. The wound had still many sloughs adherent to the surface.

Progress. Under treatment the wound rapidly cleaned and its area was considerably diminished by drawing the edges together with strapping. Later the healing process became very slow, the scar being firmly adherent to the bone in places. At the end of November the wound, then reduced to a long narrow strip, was skin-grafted with but very little success, healing, however, continued to progress and the patient was discharged on February 27th, 1917, with the wound healed and the fibula firmly united.

Bacteriological Notes.

On admission. Films showed *Streptococci*, tetrads, *Staphylococci*, and a coliform bacilli. Cultures gave a typical form of *B. welchii* and a bacillus resembling *B. cochlearis*, also *Staphylococcus* and a coliform bacillus which produced acid and gas in broths containing glucose, lactose, dulcite, saccharose, and mannite.

October 19th. Films showed many bacteria, diphtheroids, and *Staphylococci*.

October 26th. A pyo-sero culture gave pure *Streptococcus pyogenes*.

November 8th. Film showed many organisms, *Streptococci*, *Staphylococci*, coliform bacilli, and diphtheroids. Cultures gave pure *Streptococcus pyogenes*. Cultures made from heated pus remained sterile.

Case No. 35. Wounded (grenade) 13.10.16. Admitted 20.10.16. Discharged 7.3.17.

History. Operated on at a Casualty Clearing Station and again at the Base for gas gangrene.

Condition on admission (7th day). There was a very extensive wound of the left thigh about 12 inches in length commencing from the fold of the buttock down the thigh; it extended deeply into the intermuscular planes, the muscles being widely separated; there was, however, no great loss of skin. There were two other wounds on the left buttock, one of them having an area of about 5 square inches. The femur was uninjured. These wounds were cleaning, but some sloughs still remained attached. There was also a large sloughy wound on the left forearm roughly oval in shape and about six inches long extending deeply into the intermuscular tissues; the bones were not fractured. The fingers of the left hand were much contracted and flexed, being in the position of *main en griffe*; the interossei and other small muscles were much wasted; pain was complained of in the palm of the hand, thumb, index, middle, and radial side of the ring fingers. This condition was evidently due to bruising of the median nerve.

In addition there were signs of low grade arthritis in the joints of the fingers, the wrist, elbow, and shoulder joint of the right arm. The patient stated that before he had been wounded these joints were quite normal.

Progress was uneventful, the arm wound healed without any troubles developing, the healing being very much hastened by a number of Steele's grafts, all of which took and rapidly formed epithelium round them. The thigh wounds progressed very rapidly in the early stages and everything went well until the beginning of January 1917, when the surface became unhealthy and the newly-formed scar tended to break down. This condition rapidly improved with the use of wet 5 per cent. saline packs, and the patient was discharged on March 7th with all his wounds soundly healed. There was, however, still some pain in the palm of the hand and the fingers supplied by the median nerve, and in spite of careful splinting the fingers were still somewhat contracted; the chronic arthritic condition of the left shoulder, elbow and wrist, and smaller joints of the fingers still persisted, although the lesions were no longer progressive.

Bacteriological Notes.

On admission. Cultures from the thigh wound gave *B. proteus*, *Staphylococcus aureus*, coliform bacilli, *Streptococcus faecalis*, and diphtheroids. Cultures from the heated pus were sterile. Films made from pus from the wound of the forearm showed many bacteria, *Staphylococci*, *Streptococci*, and diphtheroids. Cultures from arm gave *Staphylococcus*, diphtheroids, *B. proteus*, but no colonies of *Streptococci* could be found. Anaerobic cultures gave *B. sporogenes*.

October 25th. Cultures from the thigh gave *Staphylococcus* and *Streptococcus*.

November 8th. Films from the thigh showed a very few *Streptococci*. Cultures were overgrown with *B. proteus*.

November 20th. The wound of the forearm before being grafted gave cultures of *Staphylococcus* and a diphtheroid.

December 5th. Wound of forearm—Culture pure *Staphylococcus*.

January 10th. Wounds of buttock and thigh, which were at that time not doing at all well, gave abundant cultures of *B. pyocyaneus* and *Streptococcus*.

Case No. 36. Wounded (shell) 8.8.16. Admitted 15.11.16. Transferred on closing of wards, 23.6.17.

Condition on admission (100th day). Several large healed scars were present, on the outer side of the thigh below the trochanter and over Scarpa's triangle. There was a sinus leading down to the neighbourhood of the fracture from about

the middle of Scarpa's triangle and another one also leading to the same part and opening behind the great trochanter. X-rays showed the fracture with the lower fragment drawn up behind the upper one, which was considerably flexed. The shortening of the limb amounted to 3½ inches. There were also very many small pieces of metal scattered in the tissues. The fracture was firmly united.

Progress. The condition remained practically stationary for some weeks, a good deal of difficulty being encountered in maintaining efficient drainage. Two operations were performed to promote drainage and for the removal of sequestra, with the result that the wounds were almost healed when he was discharged from hospital.

Bacteriological Notes.

On admission. Films showed *Streptococci* only. Cultures gave pure *Streptococcus pyogenes.*

December 20th. *Streptococcus pyogenes* and a few *B. proteus.*

Films and cultures were made from time to time; the cultures always gave *Streptococcus pyogenes* and on one occasion *Streptococcus anginosus* in addition. *B. proteus* and diphtheroids were also occasionally present.

Case No. 37. Wounded (shrapnel) 18.10.16. Admitted 9.11.16. Discharged 1.1.17.

History. Pieces of shrapnel were removed from left elbow and left knee at the Base, where he had a slight secondary haemorrhage from a small vessel in the right axillary fold.

Condition on admission (21st day). The patient had a clean wound about 4 inches long extending from the right anterior axillary fold down the arm. There were two small granulating flesh wounds on the front of the left arm just above the elbow joint. There was also a clean wound on the front of the right side of the chest a little below the clavicle, and two wounds on the inner side of the left knee which did not open into the joint.

Progress. The wounds on the chest, left arm, and left leg healed rapidly; the large wound on the right arm was skin grafted, which considerably shortened the patient's convalescence. The patient was discharged on January 1st, 1917, with all the wounds soundly healed.

Bacteriological Notes.

On admission. Pus from the large wound of the right shoulder and arm; films showed *Streptococci, Staphylococci,* coliform bacilli, and diphtheroids. Cultures gave growths of *B. proteus, Streptococcus pyogenes, Staphylococcus,* and diphtheroids. From the small wound on the knee only *Staphylococcus.*

November 11th. Cultures from the wound on chest gave *Streptococcus* and *B. pyocyaneus*; from the wounds near left elbow and left knee *Streptococcus pyogenes.*

Case No. 38. Wounded (bomb) 13.11.16. Admitted 21.11.16. Discharged 3.2.17.

History. He was kept at a Casualty Clearing Hospital for four days and was there operated on for commencing gas gangrene.

Condition on admission (8th day). General condition was good. There was a large, deep, irregular wound in the region of Scarpa's triangle which roughly measured 8 by 6 inches; much skin, fascia, and muscle had been lost. The surface of the wound was very dirty and many sloughs were still adhering.

Progress. This was at first extremely rapid, the wound cleaning in a very few days and at first contracting very rapidly. Later the healing process became very much slower, the scar tissue forming a hard dense mass. In the early part of January the surface was skin grafted with but little success owing probably to the dense scar tissue cutting off practically all the lymph supply. Healing, however, continued and was seemingly very distinctly assisted by vigorous massage of the mass of scar tissue, a procedure which was quite painless. The patient was discharged on February 3rd with good and powerful movements of his leg and no lameness.

Bacteriological Notes.

On admission. Films showed very many bacteria, *Streptococci* and diplococci, tetrads, small Gram-positive bacteria, some very large Gram-positive diplococci, large Gram-positive bacteria and large Gram-negative bacteria, coliform bacilli, diphtheroids, and *Staphylococci.* Cultures, anaerobes, *B. welchii, B. tertius,* and *B. sporogenes. Streptococcus pyogenes* and diphtheroids were also grown.

December 4th. Film only showed many diphtheroids and a few *Streptococci*. Cultures gave diphtheroids.

December 27th. Very few bacteria seen in films, and all were diphtheroids.

Films made from the wound on various occasions after this date never showed many bacteria, usually only one or two diphtheroids could be seen lying inside phagocytes.

Case No. 39. Wounded (shell) 13.11.16. Admitted 21.11.16. Died 26.11.16.

History. The patient was operated on at a Casualty Clearing Hospital for gas gangrene.

Condition on admission (8th day). The general condition was not very bad. He had a single large wound on the posterior aspect of the thigh roughly measuring 8 by 6 inches; this was in a very foul condition, being covered with sloughs; the skin edges were thickened and everted, the muscles were much torn and digested away, there was a considerable loss of skin and subcutaneous tissue, but parts of the wound did not involve the muscles.

Progress. Under treatment the wound began to clean rapidly and the sloughs to separate. His general condition gave no anxiety until November 25th, when the pulse rate began to rise. The wound showed no signs of developing gas gangrene, erysipelas, or any cellulitis. The patient died quite suddenly and unexpectedly on November 26th; there were no preceding signs of embolus or haemorrhage to account for the sudden death. Post-mortem gave no further indications as to the cause of death except that the heart showed slight fatty degeneration of the muscle, which was very flabby.

Bacteriological Notes.

On admission. Films showed very many bacteria, *Streptococci* and diplococci, diphtheroids, coliform bacilli, tetrads, Gram-negative diplococci, long Gram-negative bacilli, some of which had oval spores, large and small Gram-positive bacilli. Cultures, anaerobic, gave *B. welchii*, *B. sporogenes*, *B. tertius*, *B. sphenoides*, and a bacillus resembling *B. tetani*. *Streptococci* and coliform bacilli were also isolated.

Post-mortem. The heart's blood was found to be sterile.

Case No. 40. Wounded (shell) 12.11.16. Admitted 4.12.16. Discharged 11.3.17.

History. The patient remained in a Casualty Clearing Station for seventeen days and during that time was operated on six times for gas gangrene.

Condition on admission (22nd day). The patient's general condition was good, but any noise or sudden start always brought on pain in his injured leg. There was a superficial partially healed wound of the back just below the left scapula. The principal wound was situated at the back of the right thigh and extended from the back of the knee upwards for about 10 inches. There was a very extensive loss of skin, fascia, and muscles over the whole area. There was marked foot-drop and frequent attacks of pain in the outer side of the leg and dorsum of the foot evidently due to the injury to the outer part of the sciatic nerve

Progress. Under treatment the thigh wound very rapidly commenced to heal, and it was then seen that so much skin, &c., had been lost that unless some plastic operation was performed a very large area of scar tissue would have to form and healing would take a very long time. On December 20th, two flaps were dissected up, one on each side of the wound, and brought over to meet each other across the wound. This operation was successful, the flaps remaining in position and no sloughing taking place, the wounds being dressed from the earliest stages with hypertonic salt solution. On January 1st the raw areas from which the flaps had been removed were skin-grafted by Steele's method with a very successful result, a large portion of the wounds being quickly covered by epithelium. Another grafting was performed on February 17th, with equally good results on one side of the thigh, but with less success on the other side, owing to movement of the grafts. However, when the patient was discharged on March 11th, all his wounds were healed, and there was no contraction of the knee, which had full and strong movements. The periodic attacks of pain and the foot-drop were both very much improved.

Bacteriological Notes.

On admission. Films showed diphtheroids, *Staphylococci*, some Gram-negative cocci and Gram-negative bacilli, but *no Streptococci*. Cultures gave

B. pyocyaneus, diphtheroids, and Gram-negative bacilli; *Streptococci* were also grown. From the wound of the back cultures gave diphtheroids and *Staphylococci*.

December 12th. Pyo-sero-cultures gave *Streptococcus pyogenes*.

December 21st. The day after the operation, cultures gave numerous colonies of *Streptococcus* and some diphtheroids.

December 22nd. Cultures gave many colonies of *Streptococcus pyogenes* and diphtheroids.

December 27th. Films from an unhealthy-looking spot showed many bacteria, *Streptococci*, diphtheroids, and *Staphylococci*. Cultures gave *Streptococci*, diphtheroids, and *Staphylococci*.

December 28th. Heated pus cultures were sterile.

December 29th. Film showed practically no bacteria.

February 15th. Culture from the portion of the wound of thigh dressed with brilliant green was sterile, while one made from the part dressed with 'Comfrey' gave six colonies of *Staphylococcus*.

Case No. 41. Wounded (shell) 13.11.16. Admitted 15.12.16. Discharged 31.3.17.

History. At the Base, twelve days after the injury, the right leg was amputated for sepsis through the knee joint.

Condition on admission (32nd day). The patient's general condition was very fair. The skin and soft parts had contracted away from the condylar end of the femur, leaving a large granulating surface and a sinus opening on the posterior edge.

Progress. Under treatment with wet 5 per cent. saline packs and later normal saline the surface cleaned rapidly. Extension of the soft parts was applied and the granulating surface successfully skin-grafted on three occasions. The stump was soundly healed when discharged, but reamputation may have been necessary later.

Bacteriological Notes.

On admission. Films from the granulating surface of the stump showed Gram-positive cocci and diphtheroids. Cultures gave growths of *Streptococci*, *Staphylococci*, and a coliform bacillus which fermented with the production of acid and gas glucose, lactose, and mannite, but had no action on dulcite or saccharose. Films from the sinus showed many bacteria, large Gram-positive cocci, diplococci, and diphtheroids. Cultures gave colonies of *Streptococcus*, *Staphylococcus*, and diphtheroids.

January 10th. Cultures showed an almost pure growth of *Streptococcus*.

Case No. 42. Wounded (shell) 16.12.16. Admitted 29.12.16. Discharged 31.3.17.

History. Patient had flesh wounds of the left thigh, thoracic wall, and left shoulder.

Condition on admission (13th day). The patient's general condition was not satisfactory. There were at times slight gastro-intestinal disturbances with abdominal pain, also pain in the feet, and for several weeks there was some irregular fever. Four small granulating wounds were present in the left upper arm and thorax and a large lacerated wound in the lower third and outer aspect of the left thigh. This wound was also beginning to granulate, but showed a considerable amount of discharge and its surface did not look quite healthy.

Progress. The healing of the wounds was apparently much delayed by the persistent fever for which no adequate cause could be found. A blood culture was made about January 22nd with a negative result. The stools were examined bacteriologically on January 25th, and *B. dysenteriae*, Flexner, was isolated. There were no symptoms or history of true dysentery, but the patient complained of abdominal pain. Three later bacteriological examinations of the stools were all negative.

The patient began to improve rather suddenly as regards general symptoms about February 9th, and after that continued to convalesce steadily. He was discharged on March 31, 1917, with all his wounds healed and his general condition quite satisfactory.

Bacteriological Notes.

On admission. (Notes lost.)

January 10th. Cultures from the wound of the thigh gave *B. proteus*, *Streptococci*, and *B. pyocyaneus*.

January 22nd. Blood culture negative.

January 25th. *B. dysenteriae* (Flexner) isolated from faeces. This bacillus was identified by (1) its fermentation tests—it produced acid in glucose and mannite broths, but had no action on lactose, dulcite, saccharose, or maltose—and (2) by it being agglutinated to full titre by a standard serum. On February 8th, 9th, and 12th, the stools were again examined, but no *B. dysenteriae* could be found.

February 17th. A film made from the wound only showed a very few cocci.

Case No. 43. Wounded (shell) 1.11.16. Admitted 4.1.17. Discharged 23.6.17.

History. The patient's injuries were: (1) Compound fracture of the skull in the right frontal region. (2) Compound fracture of the right tibia with division of the anterior tibial nerve. (3) Flesh wounds of both legs, the chest wall, and right hand.

At the C.C.S. portions of the fractured skull bones were removed and pieces of shrapnel from his other wounds. At the Base pyaemic foci developed in the left elbow and wrist joints as well as several pressure and bed sores which also suppurated.

On admission (65th day). His general condition was bad, with great emaciation and anaemia. The temperature was constantly raised. All the wounds were suppurating freely, including the pyaemic foci which had been incised, and cerebral pulsations were very marked in the frontal wound. The left elbow especially gave occasion for anxiety.

Treatment. His general condition was treated by small doses of *Streptococcus pyogenes* vaccine which appeared to quickly control his temperature and no further pyaemic abscesses developed. Later small doses of staphylococcic vaccine were given in addition.

The left elbow was slung in a counterpoised splint made of perforated celluloid which enabled intermittent irrigation to be easily carried out.

Progress. Under this treatment the patient quickly improved; it was, however, necessary on January 19th again to incise freely the left elbow joint to establish efficient drainage, but the inflammation of the left and right wrist joint subsided without further surgical interference. After this date improvement was continuous both as regards the general condition and the healing of the various wounds. Several pieces of bone separated from the skull and were removed from time to time. On March 28th, a skiagram having indicated the presence of a sequestrum in the right tibia, the wound was opened up and the dead bone was removed. This wound then slowly healed, a considerable cavity in the bone having been made. The patient was discharged on June 23rd, 1917, his condition then being that the head wound had soundly healed but required a protecting plate as the cerebral pulsations were still very distinct. The wound of the right leg had almost completely healed and there was no indication of any necrosed bone. The left elbow had healed completely and soundly, but the joint had very little movement; the left hand had healed, but the movements of the left wrist were greatly impaired. The wounds about the right wrist had also healed, but again the movements of this joint were limited; both wrist joints were improving, however, under massage.

Bacteriological Notes.

On admission. Pus from the wound of the head. Films and cultures showed *Staphylococcus* and *Streptococcus pyogenes*. Pus from the left hand gave a pure culture of *Streptococcus*.

January 16th. Films from a membrane which had formed over the left elbow showed practically nothing but diphtheroids of the short type. In culture two types of diphtheroids grew, some of the colonies showing a short bacillus, others a longer bacillus. Neisser's stain was practically negative. Five colonies were subcultured and their fermentation reactions tested, all gave acid in glucose and saccharose broths, none of the other sugars, &c., which were tested showed any change. Cultures from the pus of the elbow joint taken on the same day gave a pure culture of *Streptococcus pyogenes*, and films made from it showed nothing but *Streptococcus*.

January 19th. Pus from the right leg. Films showed a few cocci only, Cultures gave *Streptococcus pyogenes* and *Staphylococcus*. Heated pus was sterile.

January 21st. Pus from elbow. Films showed but few bacteria, chiefly diphtheroids, but also a few *Streptococcus*.

January 31st. Cultures from pus from the head. Aerobic plate gave *Staphylococcus* only. Anaerobic broth cultures gave *Streptococcus pyogenes* and *Staphylococcus.*

February 15th. Film made from pus from the elbow showed no bacteria.

March 29th. Cultures made from the sequestrum removed by operation from the right tibia on March 28th, when grown in broth anaerobically, gave Gram-positive bacilli resembling morphologically *B. welchii*, Gram-negative bacilli, and a *Streptococcus.* Pus taken the day after the operation showed in films *Streptococci* and a bacillus resembling *B. welchii.* Cultures, aerobic and anaerobic, gave *Staphylococcus, Streptococcus pyogenes,* and a coliform bacillus.

March 30th. Cultures made from the pus (second day after the operation) gave only *Staphylococci* and *Streptococcus pyogenes.*

April 2nd. Film made from the pus (fourth day after the operation) showed *Staphylococci* and *Streptococci.* Cultures gave *Streptococcus pyogenes* and *Staphylococcus.*

April 3rd. Film from the pus (fifth day after operation) again showed *Streptococci* and *Staphylococci.* Cultures gave *Streptococcus pyogenes* and *Staphylococcus.*

April 12th. Pus from the same wound (fifteen days after operation). Films showed diphtheroids, *Staphylococci,* and *Streptococci.* Cultures gave *Staphylococcus* and *Streptococcus pyogenes.*

Case No. 44. Wounded (shell) 10.1.17. Admitted 22.1.17. Discharged 24.3.17.

History. Gas gangrene had developed round a wound in the thigh and had been treated by extensive excision of the infected tissues.

Condition on admission (12th day). There was a large clean wound on the inner side of the right thigh. There was also a counter opening for drainage on the posterior aspect. The surface was quite clean. Two days after his admission an attempt was made to close the wound by secondary suture ; so much scar tissue had, however, formed in the depth of the wound that great tension on the sutures was necessary to bring the skin edges together ; the result therefore was not very successful.

Progress. In spite of the failure of the secondary suture to close the wound, healing at first was very rapid ; later it became very much slower and skin-grafting was employed, again without much success, owing most probably to the presence of a dense mass of scar tissue beneath the granulating surface. The final result was, however, quite satisfactory, and the patient was discharged on March 24, 1917, the wound being quite healed and the movements of the thigh excellent.

Bacteriological Notes.

On admission. Films showed very few bacteria, a few *Streptococci* and coliform bacilli being seen. Cultures gave a few colonies of *Streptococcus* and a coliform bacillus.

January 29th. Cultures made after the operation were overgrown with *B. proteus.*

February 9th. Culture made from a boil which developed near the edge of the wound grew *Staphylococcus* only.

March 8th. Culture made 10 days after the grafts were applied gave *B. proteus.* Films showed no bacteria.

Case No. 45. Wounded (bullet) 10.1.17. Admitted 23.1.17. Discharged 17.3.17.

History. At a Casualty Clearing Station the wound had been thoroughly opened up.

Condition on admission (13th day). The patient's general condition was good ; the wound was situated rather to the inner side of the right thigh and penetrated through to the posterior surface, neither the bone nor any important vessel or nerve had been injured ; the anterior wound was the largest, being roughly 6 inches long and 3 inches broad ; from it two tracks passed between the muscles towards the bone. On January 29, six days after his admission the anterior wound was brought together by secondary suture, the wound having been previously treated with 5 per cent. saline packs to complete the cleaning of the surface. This was very successful, as but little tension was necessary to bring the edges together, and by treating the sutured wound from the beginning

with 5 per cent. saline packs which were kept constantly wet by means of tubes arranged in the dressing the resulting sepsis was very slight and healing was very rapid.

Later progress was quite uneventful, and the patient was discharged on March 17 completely healed and with full movements of his legs.

Bacteriological Notes.

On admission. Films showed very few bacteria which consisted chiefly of Gram-negative bacilli. Cultures gave *B. welchii*, *Staphylococci*, *B. pyocyaneus*, and diphtheroids.

February 2nd. Four days after the secondary suture of the anterior wound pus taken from the sutured portion showed in films chiefly diplococci (most probably *Streptococci*); the cultures were overgrown with *B. proteus*.

Case No. 46. Wounded (bomb) 12.11.16. Admitted 14.1.17. Died 14.2.17.

History. The left foot was amputated for gas gangrene, and as the gangrene recurred in the stump, a further amputation was performed below the knee. Pyaemia then set in, the right knee filling with pus and several other abscesses forming, one over the right tibia being subperiosteal and another inter-muscular in the right thigh. Periostitis of the left great trochanter also developed and an incision down to the bone was made in this region. A large bed-sore also formed over the sacrum. *Staphylococcus* and *B. pyocyaneus* were found in all the wounds.

On admission (62nd day). The patient's condition appeared quite hopeless. The temperature was high, the pulse rapid and irregular, and there was very marked wasting and anaemia. He was very drowsy.

The wounds of the right foot had healed, and the amputation stump was healing slowly, but both leg and thigh on the right side and the trochanteric region on the left were very widely excavated by the pus tracking from the original pyaemic abscesses, drainage was also very imperfect. An almost solid oedema was present in both legs up to the groin. Over the sacrum was a large deep bed sore.

Treatment. Treatment consisted of the removal of sloughs and the maintenance of efficient drainage from the various pyaemic abscesses.

Progress was steadily down hill; further pyaemic abscesses developed in the right thigh and leg, and an extensive septic thrombosis either of the vena cava inferior or the common iliac vein of the right side occurred. Pleurisy with effusion also developed on the left side shortly before the patient's death on February 14th, 1917. There was no post-mortem examination.

Bacteriological Notes.

Staphylococci and *B. pyocyaneus* had been isolated from the wounds at Boulogne.

On admission. Films from the wound below the right knee showed many *Staphylococci* and a few smaller diplococci which might have been *Streptococci*. Films from a slough taken from the wound over the left great trochanter showed many bacteria, principally *Staphylococcus*, *Streptococcus*, diphtheroids, and a Gram-negative bacillus. Cultures of the pus from the right knee grown aerobically and anaerobically gave colonies of *Staphylococcus* only. Cultures from the left trochanter region gave *B. proteus* and *B. pyocaneus* in addition to *Staphylococcus* and *Streptococcus*. Heated pus from the right calf was sterile.

January 17th. A blood culture remained sterile.

February 8th. Pus from a freshly opened pocket in the region of left great trochanter gave *Staphylococcus*, *Streptococcus*, and diphtheroids.

February 10th. A pyo-sero-culture from the left great trochanter gave *Staphylococcus* and diphtheroids only.

Remarks. The patient appears to have been practically the only case in which *Staphylococcus* played the most important rôle in the bacterial infection. In the secondary abscesses, which were embolic in nature, *Staphylococcus* was always present, and although *Streptococci* could usually be demonstrated they were very much fewer in number and their presence was not constant.

Case No. 47. Wounded 13.1.17. Admitted 28.1.17. Discharged 26.3.17.

History. At the Base on the 3rd day he was found to be suffering from local gas infection, which was treated by incision and 5 per cent. salt packs.

Condition on admission (15th day). The patient's general condition appeared quite satisfactory, although he had a considerable amount of fever. His wound was situated on the front and inner aspect of the right upper arm, the skin being

destroyed over a very considerable area together with extensive laceration of the muscles beneath. The main vessels and nerves were not involved, and the bone was uninjured. The wound surface was cleaning, some sloughs still adhering to the surface.

Progress. On the fourth day after the patient's admission he had a rigor and the temperature rose to over 104° F.; at the same time a rather dusky red blush was noticed round the wound, which rapidly spread from the arm to the body and then downwards over the back and side until a very considerable area of skin was involved. There were no bullae. This was at first thought to be an attack of erysipelas and small doses of vaccine made from *Streptococcus pyogenes* were administered, but the temperature remained high, 104° F. to 105° F. every evening, dropping to almost normal in the morning. The general condition remained very good, the pulse was never rapid, 96 per minute being the highest recorded and more often it was from 80 to 88. Respiration varied from 18 to 24 per minute. The rash soon began to gradually fade, the temperature becoming more intermittent from 104° F. in the evening to 96° F. in the morning. With the fading of the rash desquamation commenced from the whole area of the trunk occurring far beyond the limits of the rash. The temperature fell to normal on the eleventh day after the rigor which had ushered in this condition. During the attack there was a marked 'loss of voice', the patient only being able to speak in whispers. A blood culture taken the day after the rigor remained sterile. In many ways this attack was clinically unlike erysipelas and far more resembled the disease which has been described as dermatitis exfoliativa.

The subsequent history was quite uneventful; the granulating wound was skin-grafted on two occasions with most perfect results, a large surface of about nine square inches being completely healed in just over three weeks. The patient was discharged on March 26th, 1917.

Bacteriological Notes.

On admission. Cultures of the pus on glucose agar plates gave no growth; these plates were incubated aerobically. The appearance of the pus on the dressing indicated the presence of *B. pyocyaneus.*

January 30th. Glucose agar plates incubated first anaerobically then aerobically gave only one or two colonies of *Staphylococci.* A glucose broth tube in which was a small piece of potato after 3 days' incubation gave a culture of *Staphylococcus.*

January 31st. (The day the rash first appeared.) Culture on glucose agar plate first incubated anaerobically then aerobically gave *no* growth whatever.

February 1st. Film showed a considerable number of Gram-positive diplococci. Culture on glucose agar plate gave two colonies of *Staphylococcus* only. Blood culture negative.

February 2nd. Agar cultures aerobic and anaerobic gave numerous colonies apparently all *Staphylococcus.*

February 4th. A film of the pus shows only a few Gram-positive cocci, mostly diplococci. Culture on agar incubated aerobically gave a fair number of colonies of *Streptococcus pyogenes* and a few *Staphylococcus.*

February 23rd. Two films taken before skin grafting, one showed no bacteria, the other a very occasional *Staphylococcus.*

March 7th. Film taken before second skin-grafting operation showed no bacteria. Culture on agar gave a single colony of *B. pyocyaneus.*

The repeated failure to obtain cultures cannot be explained. The media cannot have been at fault, as numerous other cultures were being made daily on samples from the same stock. As the wounds were being dressed with saline solutions it cannot have been due to antiseptics.

Case No. 48. Wounded (bullet) 5.1.17. Admitted 28.1.17. Died 9.2.17.

History. The wound was 'excised' at the Casualty Clearing Station. At the Base the wounds progressed slowly, but the patient suffered from bronchitis and his general condition was stated not to be good.

Condition on admission (23rd day). The general condition was fair, but the patient seemed rather exhausted by the journey, and the temperature was considerably raised. His wounds were (1) a granulating area over the right calf about 4 by 4 inches extending into the muscles, parts of which were lost; (2) two dirty wounds one on each side of the lower part of the thigh just above the knee.

These wounds were about 3 inches long and opened into the popliteal space (a former note stated that the popliteal vessels were exposed in the wound). There was a considerable amount of unhealthy pus coming from both wounds.

Progress. The day after the patient's admission, severe secondary haemorrhage occurred, the inner wound was, therefore, opened up and the bleeding point sought for, but the tissues were so infiltrated with blood and inflammatory exudation that it could not be found, the leg was therefore amputated by an oblique incision, no attempts being made to close the wound. The patient rallied well after this, the stump was cleaning well and the temperature was daily taking a lower level, when on the eleventh day after the amputation a very severe secondary haemorrhage took place in the middle of the night, and the patient died within half an hour of its discovery.

Bacteriological Notes.

February 1st. Films from the stump showed enormous numbers of diphtheroids, a few chains of *Streptococci*, and some diplococci.

February 2nd. Film from the stump: Gram-negative bacilli the most numerous, but large numbers of diphtheroids, chains of *Streptococci*, and diplococci also present as well as Gram-positive bacilli. Cultures gave numerous very small colonies of a strepto bacillus, also two varieties of *Streptococci*, one of which was *faecalis*, the other *pyogenes*, and a large bacillus. Anaerobic cultures gave *B. welchii* and *Streptococcus faecalis*.

February 3rd. Film from stump shows fewer bacteria; diphtheroids and Gram-negative bacilli, are still the most numerous, the anaerobic types are much rarer, the cocci, both *Streptococci* and *Staphylococci*, are relatively increased.

February 4th. Film from stump which is cleaning, but had still a good deal of pus lying in the intermuscular planes, shows fewer bacteria, chiefly diphtheroids, Gram-negative bacilli and *Streptococci*. A film taken from near the skin edge shows comparatively few bacteria, diphtheroids and cocci only being seen.

February 5th. Films from stump. Bacteria still numerous, diphtheroids, *Streptococci*, Gram-negative bacilli, a few anaerobe-like types.

February 6th. Film from stump still shows numerous diphtheroids, *Streptococci*, and Gram-negative bacilli.

February 7th. Film shows far fewer bacteria, diphtheroids, and *Streptococci*

February 8th. Film shows still fewer bacteria, diphtheroids, *Streptococci*, *Staphylococci*, and Gram-negative bacilli were still noted. Cultuses were overgrown with *B. proteus*.

February 9th. Film shows much fewer bacteria, chiefly *Streptococci*.

Case No. 49. Wounded (shrapnel) 22.12.16. Admitted 28.1.17. Transferred 31.3.17.

History. On December 24 some foreign bodies were removed; on the 26th multiple incisions were made in the lower part of the upper arm and the upper part of the right forearm. On December 31 the elbow joint (right) was opened up and a resection was performed.

Condition on admission. On admission (38th day) the patient's general condition was good; the main wound was on the outer side of the elbow joint with which it communicated by several tracks lined with soft granulations. There were several almost healed incisions about the upper arm and forearm No swelling or signs of cellulitis.

Progress. Quite uneventful, but rather slow. The patient was discharged on March 31st, 1917.

Bacteriological Notes.

On admission. Films from the larger wound showed a few bacteria only. *Streptococci* and a Gram-negative bacilli. Cultures gave colonies of a Gram-negative coliform bacillus, a diphtheroid, *Staphylococcus*, and *B. pyocyaneus*.

January 31st. Culture gave *Staphylococcus*, diphtheroids, *B. pyocyaneus*, and *Streptococcus* of the *faecalis* type.

February 8th. Cultures gave colonies of *B. proteus*, *Staphylococcus*, *B. pyocyaneus*, diphtheroids, and a coliform which fermented glucose, saccharose, mannite and maltose producing acid but no gas and had no effect on lactose, and dulcite. It was non-motile. In broth it produced a scum on the surface and the colonies on agar were quite unlike *B. dysenteriae*.

February 15th. Cultures gave *Streptococci* and *Staphylococci* only.

Case No. 50. Wounded (shrapnel) 10.1.17. Admitted 29.1.17. Discharged 7.5.17.

History. The patient remained in a Casualty Clearing Station for 8 days and was then transferred to the Base, where he stayed for 12 days. His condition when in the Casualty Clearing Station was described as follows: He had a severe comminuted fracture of the left ulna 2 inches below its upper end. From a wound 7 inches long which commenced near the external condyle of the humerus and passed down the ulnar line of the forearm the head of the radius protruded; the bone and cartilage was quite uninjured, but the ligaments of the elbow joint were extensively torn and there was severe laceration of the extensor muscles. The posterior interosseus nerve was damaged. The wound was cleaned, the radial head was returned and sutures put in. The wounds of the left leg were excised and drained. Five days later the elbow wound had to be opened up and part of the joint capsule was cut away, the head of the radius was then returned. B.I.P.P. applied to the wound, which was then sutured.

Condition on admission (19th day). The general condition was good, the wound of the elbow had completely opened up, a few remaining sutures coming away at the first dressing; the head of the radius was lying quite on the surface of the wound, which was suppurating freely. It was decided not to perform any operation but to allow the wound to heal and, after an interval of some months, reset the joint. A wound of the left thigh was quite superficial and granulating satisfactorily.

Progress was quite uneventful and rapid; the wound was skin-grafted on two occasions with striking success. The sinus leading to the site of the fractured ulna healed rather more slowly as some small bone fragments came away. The patient was discharged on May 7th with the elbow quite healed, but the head of the radius projected markedly, the scar being adherent to it. The joint was fixed at a right angle. He was recommended to have a resection of the joint performed some months later.

Bacteriological Notes.

On admission. A film from a pustule on the thigh showed *Staphylococcus*. Cultures from the same source gave *Staphylococcus* only. Film from the elbow showed *Staphylococcus*, diphtheroids, and *Streptococcus*. Cultures gave *Staphylococcus* and diphtheroids. Cultures from heated pus gave a Gram-positive anaerobic bacillus with a subterminal spore, ? *B. sporogenes*. No subcultures were made.

February 9th. Pus from the thigh wound. Films showed diphtheroids and a few *Staphylococcus*. Cultures gave *Staphylococcus*, diphtheroids, and *B. proteus*.

February 12th. A pyo-sero culture from the elbow gave only *Staphylococcus*.

Case No. 51. Wounded (shrapnel) 11.1.17. Admitted 4.2.17. Transferred 23.6.17.

History. Multiple shrapnel wounds of the right leg and foot, the largest one involving the popliteal space and upper calf region. Here the muscles were much lacerated, the external popliteal nerve divided, the internal popliteal nerve exposed. After cleaning up the wounds at the C.C.S. the divided nerve was sutured, but, owing to sepsis, the sutures cut out after a few days.

Condition on admission (24th day). The patient's general condition was good. There were several very small wounds of the right foot which were almost healed and a small superficial wound on the outer side of the calf. The large wound was cleaning, but there was much loss of skin, so that the whole popliteal space was a granulating surface. There was no power to flex the ankle or extend the toes; periodical attacks of pain down the leg and in the foot were frequent and very troublesome.

Progress was very slow, the wound surface was constantly becoming unhealthy in patches and after scar tissue had formed it would here and there break down. On May 25th skin-grafting was attempted, about 50 per cent. of the Steele's grafts being successful and forming a very good scar. There was some little trouble to keep the knee extended. When transferred on June 23rd, the wound had very nearly healed, but there was marked foot-drop and loss of sensation corresponding to the distribution of the external popliteal nerve. The paroxysmal pains in the leg were much better but still occurred at times.

Bacteriological Notes.

On admission. Films from the large wound showed *Streptococci* and diplo-

cocci. Cultures gave *Streptococcus pyogenes* (mannite fermenting type), *Staphylococcus*, and a few *B. proteus*. No anaerobes could be grown.

February 7th. Film from large wound showed many cocci.

February 8th and 9th. Films from the same wound showed a few cocci only.

February 10th. Two films taken from different parts of the wound, one showed very many cocci, the other very few.

February 15th. Films from different parts of the wound all showed cocci and diphtheroids. Culture gave mostly colonies of *Streptococcus* and a few *Staphylococcus*.

February 22nd. Films showed only a few cocci.

February 27th. Surface of wound much healthier looking. Films showed very few cocci.

March 19th. Film from a wound just above the ankle showed chiefly *Streptococcus*.

Case No. 52. Wounded (shrapnel) 25.1.17. Admitted 10.2.17. Transferred 23.6.17.

History. Multiple shrapnel wounds of the left arm, leg, and foot, one also of the abdominal wall, and one of the right leg. Several pieces of shrapnel were removed from the left leg at the C.C.S. At the Base the left great toe was amputated and numerous incisions made to combat cellulitis of the leg, forearm, and upper arm. The left ankle was opened up and pieces of shattered bone removed.

Condition on admission (15th day). The patient's general condition was fair, but his temperature was raised, and he showed a great deal of nervous apprehension and restlessness. There were some twenty-five wounds altogether and practically all of them suppurating freely. Of these one, about the size of a shilling, was on the abdomen, five or six were on the left arm, and the remainder on the left lower limb. The foot was much swollen, with pus tracking downwards; between the metatarsal bones from the site of the toe amputation the ankle joint was severely infected, and the leg muscles much dissected up. There was no infection of the knee or the elbow joints, nor were there any complete fractures of the long bones. Skiagram showed very numerous foreign bodies in the left thigh, leg, and foot.

Treatment. The leg was by far the most troublesome to treat, as it was extremely difficult to maintain free drainage of the many suppurating pockets which had resulted from former incisions. A special trough splint was made from a stout sheet of perforated celluloid which, after having been softened in 5 per cent. carbolic acid, was moulded to the requisite shape on a Thomas frame, the foot being kept at a right angle. By this arrangement it was possible to carry out intermittent irrigation of the many wounds through Carrel tubes, 3 per cent. salt solution being employed at first and, later, normal saline or dry dressings.

There was for many weeks a most obstinate pustular condition of the skin of the leg and thigh, due apparently to *Staphylococcus*. Inoculation of staphylococcus vaccine failed to control the condition, but applications of a solution of flavine appeared to have a very beneficial action.

Two operations were performed on the leg and one on the arm to promote better drainage, and ultimately, after somewhat slow progress, the patient was transferred with most of the wounds soundly healed, his temperature normal, and general condition enormously improved.

Bacteriological Notes.

On admission. Pus from the *forearm*. Films showed many *Streptococci*. Cultures gave a pure culture of *Streptococcus pyogenes*. Heated pus was sterile. Pus from the *abdominal wound*. Films showed no bacteria. Pus from the wounds round the ankle. Films showed very large numbers of bacteria, diphtheroids, large Gram-positive diplococci, and *Streptococci*. Cultures gave *Streptococcus pyogenes* and *Staphylococcus*. Heated pus gave a long Gram-positive bacillus with a terminal oval spore which grew aerobically and anaerobically, and a heat resisting *Streptococcus*.

February 11th–12th. Culture from the wound of the knee gave a pure growth of *Streptococcus pyogenes*. Pus from the stump of the great toe. Film showed many *Streptococci* and some *Staphylococci*. Cultures gave colonies of *Staphylococcus* and *Streptococcus*. Pus from the knee wound. Cultures from heated pus were sterile.

February 15th. The large Gram-positive spore-bearing aerobe was again isolated from pus from the ankle joint. Films showed fewer bacteria than on the 11th, only diphtheroids and *Streptococci* being seen.

February 18th. Pus from a small sinus which opened about this date near the right ankle joint showed in films large numbers of *Streptococci*. Cultures gave *Streptococci* and *Staphylococci*.

March 9th. Pus from a fresh opening about the middle of the leg. Films showed only *Streptococci*. Cultures gave a practically pure growth of *Streptococcus pyogenes*.

March 10th. A small opening on the back of the thigh discharged pus in films which showed *Staphylococcus* and *Streptococcus*.

March 29th. Pus from the ankle. Films showed only *Streptococcus*. Cultures gave colonies of *Streptococcus* and a coliform bacillus.

April 10th. Pus from the right leg. Plates were overgrown with *B. proteus*.

April 17th. Film from a sinus about the middle of the right leg showed diphtheroids and *Streptococcus*. Cultures from the acne-like pustules of the skin of the leg gave pure *Staphylococcus*.

April 24th. A small collection of pus which formed in the left upper arm. Film showed only a few *Streptococci*.

Case No. 53. Wounded (shell) 9.2.17. Admitted 22.2.17. Transferred 23.6.17.

History. At the C.C.S. the adductor longus and semi-membranosus muscles were excised and several large incisions made for the treatment of gas gangrene.

Condition on admission (13th day). The patient's general condition was fair. There was a large irregular wound of the left thigh which commenced just below the gluteal fold and passed down the whole of the posterior aspect of the thigh as far as the bend of the knee. There was also a smaller incision wound about 8 inches long lying to the inner side of the larger wound, and a shallow oval wound about 2 inches in its long diameter just below the great trochanter. The largest wound had several tracks passing under the skin edge and often penetrating deeply into the tissues; these were not easily seen and it was only by searching carefully with a probe that they could be discovered. The general surface of the wound was clean and granulating satisfactorily.

Progress. Progress was slow owing to the size of the wound and to the presence of the irregular tracks mentioned above. On several occasions the newly developed epithelium and scar tended to break down in patches. The wound was skin-grafted with partial success on two occasions. Finally the result was most satisfactory, all the wounds being healed when he left the hospital. Movements of the leg were perfect.

Bacteriological Notes.

On admission. Films made from pus from the finger showed numerous bacteria, *Streptococci*, *Staphylococci*, diphtheroids, and coliform bacilli. Cultures from the finger were overgrown with *B. proteus*, but *Staphylococci* and *Streptococci* were also present. Films made from the surface of the thigh wound showed many *Streptococci* and also many large bacilli resembling the anaerobes. Cultures gave *Streptococcus pyogenes* and a bacillus resembling *B. welchii*.

February 24th. Films from pus obtained from a sinus leading from the large wound showed nothing but *Streptococci*. Cultures from pus on the inner side of the thigh gave colonies of *B. pyocyaneus*, diphtheroids, and *Staphylococcus*. The *B. pyocyaneus* was atypical, giving a brownish opaque colony on agar, the culture medium becoming green. This bacillus fermented glucose producing acid, but had no action on lactose, dulcite, cane sugar, or mannite.

Case No. 54. Wounded (bullet) 29.1.17. Admitted 24.2.17. Transferred on closing of wards 23.6.17.

History. On arrival at the Base on the third day, gas-gangrene developed which was treated by very extensive incisions and excision of some of the adductors and the vastus externus. Owing to the extent of the gas infection it was not found practicable to apply extension to the leg. A bed sore developed over the sacrum.

Condition on admission (26th day). The patient's general condition was not very good; pain, constantly raised temperature and loss of sleep were beginning to show their effects. There was considerable wasting. The femur was fractured at the junction of the upper with the middle third, the upper fragment being tilted forward and adducted so that it could be felt under the skin at the apex

of Scarpa's triangle. There were six large deep wounds round the left hip joint and over the left buttock, also some incisions down the front and inner side of the thigh. Posteriorily was a large deep wound leading forwards to the fracture; this was occupied by a large size drainage tube. There was also a deep ulcer over the left buttock near the middle line which had the appearance of a deep sloughing bed sore. The patient suffered much from paroxysmal attacks of pain due most probably to some injury to the sciatic nerve.

Treatment. A special splint was made by which the leg was slung with the hip joint and knee joints bent so as to bring the ends of the bone as much as possible into line, the limb being slightly abducted. Extension was applied to the lower fragment both from above the knee and from the leg.

Progress. For the first seven weeks the temperature remained high, efficient drainage being very difficult as pus was frequently being pent up. This condition was becoming serious, the patient beginning to show signs of exhaustion, when after three small doses of pyocyaneus vaccine had been administered in addition to a vaccine of *Streptococcus pyogenes*, the temperature fell rapidly to normal, where it remained for the rest of his stay in hospital. With the fall in temperature the general condition improved very rapidly, but the anterior wounds, after having almost completely healed, again broke down superficially. Healing was, however, again rapid, and when the patient was transferred on the closing of the wards the bone had united soundly in a very fair position, but with about 2½ inches of shortening, and all the wounds were healed except two sinuses, one leading to the fracture from the anterior surface, the other leading to the same site from the posterior surface. The attacks of pain over the area supplied by the sciatic nerve, which were so troublesome on his arrival, gradually became fewer and less severe.

The patient has since been seen (December 1917), when he was walking without any apparent lameness and appeared to have completely recovered.

Bacteriological Notes.

On admission. Cultures from the large wound in the back of the thigh gave colonies of *Staphylococcus*, *Streptococcus faecalis*, *B. pyocyaneus*, and *B. proteus*. Anaerobic cultures gave *B. welchii*.

March 14th. Film of the pus from the large posterior wound showed many *Streptococci* and diphtheroids.

March 27th. Film of the pus from the posterior wound showed many bacteria, coliform bacilli, *Streptococci*, and other cocci. Cultures gave colonies of *Staphylococci*, *Streptococci*, diphtheroids, and *B. proteus*.

April 3rd. Films from the same wound showed very many bacteria, *Staphylococci*, *Streptococci*, diphtheroids, and a coliform bacilli. Cultures gave colonies of *Staphylococci*, *Streptococci*, *B. pyocyaneus*, and a coliform bacillus.

April 17th. Films from the same wound still showed many bacteria ; diphtheroids, *Staphylococci*, *Streptococci*, and coliform bacilli.

May 1st. Films from the same wounds showed a moderate number of bacteria, chiefly diphtheroids, but *Streptococci* and a coliform bacillus were also present.

Case No. 55. Wounded (shell) 23.1.17. Admitted 27.2.17. Transferred 23.6.17.

History. Patient had a very severe compound comminuted fracture of the right tibia and fibula, great laceration of the muscles, and complete division of the anterior tibial nerve. On arrival at the Base on the eighth day the wound was described as ' very dirty and pouring pus ' and amputation was very seriously considered. Two large counter-openings for drainage were made on the outer and posterior aspects of the leg, and in the following three weeks there was some improvement ; but the condition became complicated by a very large pressure sore on the dorsum of the foot, which quite prohibited the use of an efficient extension.

Condition on admission (35th day). Patient's general condition was good. The large anterior wound was discharging very freely and the tibial fragments lying exposed and overriding badly. There were several very large infected bullae on the foot and ankle ; the whole leg was oedematous.

Treatment. Treatment was at first directed towards the improvement of drainage and the lessening of the suppuration ; the limb was supported in a splint lined with perforated celluloid moulded to the shape of the limb, and the wounds were dressed with 5 per cent. saline packs. Only slight extension could be

applied from the sole of the foot owing to the presence of the pressure sore on the dorsum.

Progress. Under this treatment the patient improved both in general condition and also as regards the amount of suppuration, but there was no sign of any return of function of the nerves, and it was found quite impossible to bring the ends of the bone into good position without extensive operative interference. Amputation was, therefore, performed on April 13th, the bones being divided at the level of the tuberosity of the tibia and two short lateral flaps being made ; the stump was left almost open, only two or three sutures being put in to close the vertical incisions. The wound was then dressed with 5 per cent. saline which was frequently renewed through tubes buried in the dressings. Healing was rapid and the constitutional disturbance very slight, the temperature becoming normal on the sixth day after the operation. Later a few skin-grafts were applied. The patient was transferred on June 23rd, the stump being completely healed and well covered.

Bacteriological Notes.

On admission. A film of the pus of the anterior wound showed chiefly *Streptococci.* Cultures were overgrown with *B. proteus.*

March 1st. Film of the pus collected in the anterior wound showed enormous numbers of *Streptococci* ; some *Staphylococci* and diphtheroids were also present. Cultures were overgrown with *B. proteus.* No anaerobes were found.

March 3rd. Cultures of the pus from the site of the fracture gave *Streptococcus, Staphylococcus,* and a Gram-negative bacillus, most probably *B. proteus.*

March 8th. A film of the pus still showed large numbers of bacteria ; diphtheroids most numerous but *Streptococcus* and *Staphylococcus* also present. Cultures gave *B. proteus, Staphylococcus,* and *Streptococcus.* Films were made frequently after the amputation, the discharge being taken from the surface of the stump.

April 16th. Films showed very large numbers of bacteria, the most numerous being diphtheroids ; but *Streptococci* were also abundant.

April 18th. A very large number of bacteria present in the films, Gram-negative bacilli, diphtheroids, and cocci.

April 19th. There was a great reduction in the number of bacteria seen in the films ; Gram-negative bacilli were the most numerous, but there were also many diphtheroids and a few *Streptococci.* Culture of the pus diluted 1 in 1,000 gave numerous colonies of *B. proteus* and *B. pyocyaneus* and an occasional colony of a diphtheroid bacillus. The temperature fell markedly on April 20th, and became normal on the 21st.

April 21st. Not so many bacteria were present in the films ; diphtheroids the most numerous, *Streptococci* also present. Cultures from pus diluted 1 in 1,000 gave colonies of *B. pyocyaneus* and *Staphylococcus.*

April 23rd. Culture from undiluted pus gave colonies of *Streptococci, B. proteus,* and *Staphylococcus.* Culture made from pus *diluted 1 in 500* gave no growth.

April 24th. Bacteria were present in films only in small numbers, a few *Streptococci* and long diphtheroids being seen.

May 8th. Film from a membrane that had formed on the end of the stump showed a moderate number of cocci.

May 24th. Film from the stump showed a few diphtheroids and cocci.

Case No. 56. Wounded (bullet) 25.1.17. Admitted 25.2.17. Transferred 23.6.17.

History. Bullet wound of left leg carrying a very severe compound comminuted fracture of tibia and fibula but without injury to large vessels or nerves. The leg was freely incised for gas gangrene both at the C.C.S. and also on the 6th day at the Base.

Condition on admission (31st day). The patient's general condition was only fair. There were several large suppurating wounds on the leg. The fragments of both bones were overriding but union had already begun, so that the position could not then be corrected.

Treatment. Drainage was carefully maintained ; the wounds were at first dressed with 5 per cent. saline packs, later with normal saline. The limb was slung in a perforated celluloid trough on a Thomas frame.

Progress. Under this treatment the patient progressed slowly but at first

steadily, the discharge becoming less, the swelling being much reduced, and the wound healing steadily. Firm union also occurred. At the end of March the patient had a mild and very local attack of erysipelas, after which improvement was very rapid, and this continued until May 11th, when he developed the following symptoms:

May 11th. On this day he noticed commencing stiffness of the jaws: he had had a molar extracted about one month previously; this was followed by a certain amount of inflammatory reaction which had completely subsided.

May 12th. His jaws became stiffer so that swallowing was difficult, for instance fluids could be swallowed but not solids. He complained of sore throat, but on examination nothing could be found. Seen at 10 p.m. he could open his mouth about one inch. Abdominal muscles were definitely rigid, but no weakness or rigidity of the limbs could be discovered. He complained of a feeling of stiffness at the back of the neck and in the back, but examination did not support these sensations. Twice during the evening the injured limb became drawn up. Sweating was considerable and he had an anxious expression, but his mind was quite clear and there was no mental irritability. His temperature was abnormal. Thinking the condition might have been due to commencing tetanus, at 10.30 p.m. 15 c.c. (3,000 units) of antitetanic serum (Lister Institute) were injected intrathecally after 15 c.c. of the cerebro-spinal fluid had been drawn off. The cerebro-spinal fluid was sterile and showed no cells.

May 13th. Two hours after the injection of serum the patient complained of severe headache and vomited two or three times. At 6 a.m. he became unconscious and remained in this condition until the next day. His temperature was 102° F. At 9 a.m. violent movements commenced and continued at intervals; when quiet he appeared to be sleeping. When seen at 9 a.m. he was lying on his left side with his head retracted and definitely rigid, making rotary movements with his right arm. Later the convulsions became general so that by midday he had to be forcibly restrained. *Risus sardonicus* was definitely present in the morning. He also suffered from retention of urine.

May 14th. He continued extremely violent during the night of the 13th–14th, but was calmer on the morning of the 14th. His neck muscles were more rigid. Towards the evening consciousness began to return.

May 15th. He was much better and slept for longer periods; his temperature was normal. Later he quickly recovered, the attack leaving no signs either mental or physical.

He had had two former doses of antitetanic serum, one on January 26th or 27th and the other on February 21st. During his attack a plate of false teeth in a very foul condition was found in his mouth and removed; his teeth were very decayed.

Sir Frederick Andrewes, who saw the case during the attack, wrote concerning it: 'I regard the condition as one of hysteria, verging on hystero-epilepsy, but what relation it had to the intrathecal dose of serum I do not know—the diagnosis of tetanus becomes more shaky.'

After this attack the patient progressed favourably, the amount of discharge from his leg becoming very small; skiagrams, however, showed several pieces of necrosed bone which would require removal. The bones had united firmly and the external deformity was not at all marked.

Bacteriological Notes.

On admission. Films of the pus showed chiefly *Streptococci*, but *Staphylococcus* was also present. Cultures gave colonies of *Streptococcus pyogenes*, *Staphylococcus*, and *B. welchii*.

March 7th. Cultures gave colonies of *Streptococcus* in large numbers and a few of *Staphylococcus*.

Case No. 57. Wounded (shell) 4.1.17. Admitted 27.2.17. Transferred 23.6.17.

History. The patient was wounded in the head, left shoulder, and both feet on January 4th; he remained at a Casualty Clearing Station for 4 days where some of the wounds were excised and some pieces of shrapnel were removed. He developed gas gangrene at the Base 4 days after injury on January 8th; here he was found to have gas gangrene in the neighbourhood of the right ankle and a slight infection of the left ankle. X-rays showed the bones of both feet to be uninjured; the wounds were freely opened up. On February 8th a large piece of shell was removed from the left shoulder and this wound was drained

by a counter-opening. On February 12th he was trephined and a foreign body was removed from the temporal region. The dura mater was not opened. At the same time the right ankle joint was again widely opened up.

Condition on admission (54th day). The patient's general condition was fairly good, there being but very little fever. On the left side of the scalp in the temporal region were 3 sinuses leading down to the fracture in the skull; suppuration was abundant and the scalp was very boggy and the drainage not very efficient. No dead bone could be felt. There were no cerebral symptoms. Over the left shoulder there were several small wounds which had almost healed. There was also a small suppurating wound over the point of the left elbow. The right ankle joint had been freely opened up by lateral incisions which were discharging freely, and the drainage appeared to be fairly satisfactory; but the foot and the lower part of the leg were rather swollen. Movements of the joint were very painful. Round the left ankle incisions had also been made, but these did not appear to have opened the joint.

Progress. Progress was very satisfactory although not very rapid; the swelling over the area of the injury to the skull required incising, and improved rapidly after the removal of a buried drainage tube. When transferred on June 23rd all the wounds were practically healed, one small sinus still persisting in the scalp; the wounds round the ankle joint had healed but the movements of the right joint were considerably impaired.

Bacteriological Notes.

On admission. Films of the pus from the head showed *Streptococci* and *Staphylococci*. Cultures gave *Streptococcus* and *Staphylococcus*. Film of the pus from the ankle showed *Staphylococcus* and *Streptococcus*. No anaerobes could be found.

March 16th. Film of the pus from the head showed only *Staphylococcus*. Culture gave *Staphylococcus* only. Film of the pus from the right ankle showed practically no bacteria. Cultures gave colonies of a large coliform bacillus, *Streptococcus*, and *Staphylococcus*.

Case No. 58. Wounded (mine explosion) 3.2.17. Admitted 4.3.17. Transferred 23.6.17.

History. At the Base the right leg was amputated in the lower third as dry gangrene of the foot had supervened on a Potts fracture. This was followed by some gas gangrene of the stump, which had to be incised freely. The condition of the left leg was as follows: A simple impacted fracture of the tibia just below the knee joint; the fibula was also fractured high up. The great toe and the two toes next it were gangrenous. At one time there was marked tense oedema of the left leg, but this subsided without treatment.

Condition on admission (29th day). The general condition of the patient was not at all good, there being marked disturbance of the nervous system. During the day he was morose and as a rule quiet, but in the night he was sleepless, uneasy, the condition resembling a commencing attack of delirium tremens. This condition became rather more marked, so that it was found necessary to remove him to an isolation ward for about 10 days; after this period his general condition steadily improved and his nervous system became quite normal.

The stump of the right leg, which had been amputated about 3 inches above the ankle joint, was still very septic and had some sloughs adhering to the surface; the tibia was projecting from the granulating surface; the end of the fibula was, however, covered. There were several linear incisions, partially healed, leading upwards from the granulating surface. The surface was intensely tender.

The left leg was much shortened and the upper portion below the knee a good deal deformed. X-rays showed a fracture of the tibia, the lower fragment being firmly impacted into the cancellous tissue of the head of the bone. No fracture of the fibula could be made out. The patient complained of great pain which came on at frequent intervals and passed down the leg from the seat of the fracture. The great toe and the two toes next to it were the seat of dry gangrene, and a line of demarcation was forming.

Progress. On March 30th, a distinct line having formed below the gangrenous tissues, the three toes were amputated, the head of the metatarsal bone of the great toe being saved, although the amount of skin available for a flap was very limited. These wounds were dressed with 5 per cent. saline packs immediately

after the operation. The stump of the right leg healed very slowly, but the process was very considerably hastened by skin-grafting on three occasions.

The ultimate result was very fairly satisfactory; the wounds remaining after the toes had been amputated healed soundly, leaving a very good foot. The stump was almost completely healed and the bones were covered over, a ring of bone having separated from the tibia. The patient's nervous system had become quite normal. He was transferred on June 23rd, 1917.

Bacteriological Notes.

On admission. Films of the pus from the toes showed a few *Staphylococci* and *Streptococci* and many diphtheroids. Cultures were overgrown with *B. proteus* and *B. pyocyaneus*. Films of the pus from the stump showed enormous numbers of bacteria, mostly *Staphylococci* and *Streptococci*. Diphtheroids were also very numerous. A few Gram-negative bacilli were also seen. Cultures were overgrown with *B. proteus* and *B. pyocyaneus*, so that the other varieties could not be ioslated.

March 7th. Films of the pus from the stump, bacteria not nearly so numerous: *Staphylococci*, *Streptococci*, Gram-negative bacilli, and diphtheroids. Cultures gave colonies of *B. proteus*, *Streptococcus*, *Staphylococcus*, and diphtheroids.

March 27th. Films of the pus from the stump showed *Staphylococci*, *Streptococci*, diphtheroids, and Gram-negative bacilli. Cultures gave colonies of *Staphylococcus*, *Streptococcus*, and diphtheroids.

April 5th. Films of the pus from the stump showed only a few bacteria, which consisted chiefly of Gram-negative bacilli. Cultures gave only *B. proteus*.

April 20th. Cultures gave colonies of a few *B. proteus* and diphtheroids.

Case No. 59. Wounded (bullet) 15.2.17. Admitted 11.3.17. Discharged 5.5.17.

History. At the Casualty Clearing Station he was operated on for gas gangrene, the wound being widely excised, clothing and other foreign bodies removed.

Condition on admission (24th day). The patient's general condition was good. He had three wounds on the left thigh, two of which consisted of linear incisions which were practically healed, and the other of a large wound situated on the anterior and inner surface. This wound was about 15 inches long and 5 broad; there was considerable loss of the skin, fascia, and much muscular tissue; there were a few sloughs of fascial tissue still adhering to the surface. There was a good deal of pus confined under the skin edges on the outer side of this wound.

Progress. At first the wound cleaned rapidly, so that after the patient had been treated for about 10 days the upper part of the wound was closed by secondary sutures. This was accomplished without much tension on the sutures, and free drainage was maintained by opening up a short section of one of the linear incisions. The wounds were dressed with 5 per cent. saline packs and the result was excellent, the sutures holding; and the upper portion of the wound healed completely in a very short time. Three weeks later the remaining granulating surface was skin-grafted with striking success, the whole wound being soundly healed three weeks later, and the patient was discharged on May 5th with perfect movements of the leg.

Bacteriological Notes.

On admission. Films of the pus taken from the surface of the exposed muscle showed no bacteria. Those taken from the pus collected under the skin edge showed a moderate number of *Streptococci* and diphtheroids. Cultures gave colonies of *B. proteus* and *Staphylococci*.

March 18th. Films from the upper part of the wound showed only a few diplococci.

March 19th. Cultures gave *Streptococci* and *B. proteus*.

April 10th. No bacteria could be seen in films. Cultures gave *Staphylococcus* only.

Case No. 60. Wounded (shell) 4.3.17. Admitted 11.3.17. Discharged 23.6.17.

History. No information could be obtained of his earlier treatment.

Condition on admission (7th day). The patient's general condition was fairly good. His wounds consisted of (1) a ragged tear of the left great toe, the nail and terminal phalanx being partially severed. This wound was rather unhealthy. (2) A large wound on the back of the right thigh, just above the

knee, measuring roughly 8 by 6 inches. The skin, fascia, and muscles in this region had been extensively destroyed. The sciatic nerve had also been slightly injured, paroxysmal attacks of pain being frequent and very troublesome. The surface was mostly clean but no granulations had formed, and in places sloughs were still adherent.

Progress. At first the wound cleaned rapidly and granulations formed, but the growth of epithelium and contraction of the surface were very slow. He was skin-grafted on five occasions with somewhat disappointing results; but the ultimate result was satisfactory, as when he was transferred only an area about the size of a postage stamp remained uncovered with epithelium. There was some little trouble in keeping the knee from becoming flexed, but when discharged he could fully extend the joint.

Bacteriological Notes.

On admission. Films made from the discharge taken from the clean surface of the wound showed only a few bacteria, *Staphylococci* and *Streptococci*: Films made from the pus that had collected showed large numbers of bacteria, *Streptococci*, *Staphylococci*, Gram-negative bacilli, tetrads (Gram-positive), and a few diphtheroids. Cultures gave colonies of *Streptococcus*, a coliform bacillus having the fermentation reactions of *B. coli communis* and *Staphylococcus*. Anaerobic cultures gave in addition a Gram-positive bacillus resembling *B. welchii*. The *Streptococcus* which was isolated did not belong to any classified group, but had the following characteristics: Individual cocci were round and regular, the chains were long, in broth cultures a granular deposit was formed, the supernatant broth being clear. Lactose and saccharose were fermented, acid being formed, but raffinose, mannite, and salicin were unaffected.

March 12th. Films of the pus from the left great toe showed very numerous bacteria.

March 13th. Films from the surface of the large wound showed very few bacteria, only an occasional coccus being seen.

March 17th. Film from the surface of the large wound showed only a few *Streptococci*.

March 18th. Cultures gave colonies of *Staphylococcus*. Films showed a few cocci only.

March 19th. Anaerobic cultures gave *Streptococcus* and *Staphylococcus*.

April 13th. Films show no bacteria. Cultures gave a few colonies of *B. pyocyaneus* and diphtheroids.

April 30th. Surface rather unhealthy looking. Culture gave many colonies of a coliform bacillus having the following characteristics: Motility marked. It fermented with the production of acid only glucose, and to a very slight degree saccharose. Lactose, dulcite, and mannite were unaffected.

May 3rd. After two days' treatment with flavine 1 in 2,000, during which period much of the new epithelium had digested away, films showed a fair number of *Streptococci*. Cultures gave an almost pure growth of a *Streptococcus*, having the characters of that isolated on admission.

May 8th. A film showed a moderate number of diplococci.

May 11th. After the wound had been dressed with brilliant green for 4 days, film shows practically no bacteria. Cultures gave a few colonies of *Staphylococcus* and a Gram-negative bacillus.

May 24th. Films showed a few *Streptococci*. Cultures gave colonies of *B. pyocyaneus* and a coliform bacillus with similar characters to that isolated on April 30th.

May 26th. Films showed very few bacteria. Culture gave a few colonies of *B. pyocyaneus*.

Case No. 61. Wounded (shell) 20.2.17. Admitted 9.4.17. Transferred 23.6.17.

History. At the Base the patient developed a psoas abscess, which pointed below Poupart's ligament and which was opened, a pint of pus containing *Streptococci* being evacuated. He had a previous history of having had his left testicle removed 6 years previously.

Condition on admission (47th day). The patient was very wasted and anaemic. There was a severely comminuted fracture of the humerus in the upper third of the shaft, with a wound roughly measuring 3 by 3 inches at the bottom of which lay the exposed bone. This wound was fairly clean but was discharging freely.

On taking the arm off the Thomas splint in which he had travelled, it was found that there were very extensive pressure sloughs about the left hand; both the thenar and hypothenar eminences were deeply destroyed; there was also an abscess pointing in front of the wrist. Just below Poupart's ligament on the left thigh was a sinus which passed up towards the psoas region. X-rays showed the sinus, when injected with bismuth, to be somewhat tortuous and branched; but the abscess appeared to be draining well as the patient had no temperature.

Progress was fairly rapid, the hand cleaned and healed very quickly, the fractured humerus united in good position after several pieces of bone had been removed, and the wound healed. The psoas abscess was still discharging when the patient was transferred on June 23rd.

Bacteriological Notes.

April 10th. Pus from the psoas abscess showed in films a few cocci. Cultures gave a few colonies of *Streptococcus*. Pus from the shoulder. Films showed *Streptococci* and *Staphylococci*. Cultures gave colonies of *Streptococcus pyogenes*, *Staphylococcus*, and *B. proteus*.

April 11th. Pus from the hand. Films showed *Staphylococci* and *Streptococci*. Cultures gave colonies of *Staphylococcus* and *Streptococcus*.

April 17th. Pus from the hand. Films showed only a few diphtheroids.

May 10th. Pus from the fractured humerus showed many bacteria, *Streptococci*, Gram-negative bacilli, and diphtheroids. Cultures gave *B. pyocyaneus* and *Staphylococcus*. Pus from the psoas abscess. Films showed a few *Staphylococcus* and *Streptococcus*.

May 26th. Pus from the fractured humerus—a portion of the scar had suddenly broken down. Film showed a few Gram-negative bacilli and some cocci. Culture gave large numbers of *B. pyocyaneus*.

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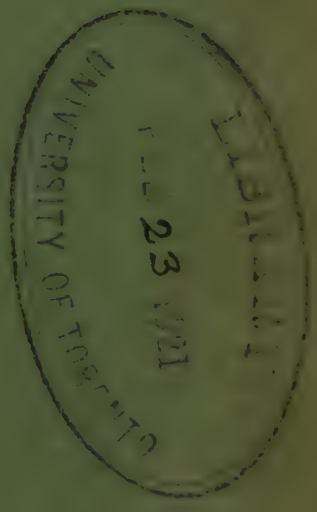
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MEDICAL RESEARCH COUNCIL

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INTRODUCTION

The occurrence during the War of serious morbidity and mortality, associated with a definite syndrome of symptoms, among munition workers who came into contact with the high explosive trinitrotoluene (T.N.T.) threatened at one time to produce a serious curtailment in the output of munitions. The earlier history of the outbreak, and of the measures set on foot to deal with it, is given in the first memorandum of the following report by Dr. W. J. O'Donovan, who relates how, when the main practical problem had been solved with a large measure of success, the Medical Research Committee, at the request of the Ministry of Munitions, appointed a scientific committee, whose function should be to gather such information, obtained in the course of the general investigation, as seemed likely to have permanent scientific value, and to promote such further investigations as would establish knowledge of the poisonous action of T.N.T. on a firmer scientific basis.

The following report, in addition to the general historical and clinical account by Dr. O'Donovan, includes papers by Dr. G. A. Wyon on the pharmacology of experimental T.N.T. poisoning; by Mr. Webster and by Dr. Dale on the metabolism and fate of T.N.T. in the animal body; and by Dr. Panton on the pathological changes produced by T.N.T. in animals experimentally poisoned and in the human victims of factory poisoning. These papers complete the series begun by the separate issue of Dr. Benjamin Moore's report (Special Report Series, No. 11). The different memoranda were drawn up by their authors at different dates and to some extent independently of one another. They will be found at a few points to overlap, and occasional redundancies and minor discrepancies were inevitable in the circumstances. It has been considered desirable to publish the memoranda as they stand, rather than obtain uniformity at the cost of the delay entailed by communicating with now scattered contributors.

Dr. O'Donovan approaches the subject from the historical, technical, administrative, and clinical aspects, and his memorandum contains an account of the rise and fall of T.N.T. poisoning. He describes how collaboration between research workers, administrators, medical men, and engineers, established such a grip upon the position, that, while in the twelve months from August 1, 1916, to July 31, 1917, there were 238 cases of toxic jaundice with 75 deaths, there were during 1918 only 34 cases with 10 deaths, even though in the last year of the war the total number exposed to risk had largely increased. This part of the report is of interest, not only historically, but as an example of combined action for a common end. Dr. O'Donovan discusses the possibility of an unknown factor, whether due to some intercurrent infection, or to some peculiar lowering of resistance, playing a necessary part in determining toxic jaundice among those exposed to T.N.T. One case quoted wherein toxic

(2332). Ps. 1460. Wt. 16632. 248. 1250. 1/21. O.U.P. A 2

jaundice seems to have been precipitated by traumatism seven months after exposure to T.N.T., is reminiscent of the part sometimes played by traumatism in determining an attack of pneumonia.

Dr. Wyon's memorandum gives clear evidence of the difficulty experienced in producing, in experimental animals, a type of T.N.T. poisoning comparable to that seen in man, and a similar experience is recorded in Dr. Panton's first paper. Interesting conclusions emerge nevertheless from Dr. Wyon's work. It will be noted, for example, that the cat, which shows very much greater sensitiveness to the poisonous action of T.N.T. than the rabbit, does not excrete in its urine any of the chromogenic substance which appears in the urine of the rabbit, monkey, or man absorbing T.N.T., and which has been regarded as the characteristic end-product of the substance, and as an indication of danger when detected in the urine of factory workers. Suggestive in this connexion is the observation recorded by Dr. O'Donovan, that patients suffering from toxic jaundice, due to T.N.T., do not show the colour reaction in the urine, even when a dose of T.N.T., sufficient to produce a clear reaction in the normal person, is rubbed into the skin. From considerations of a quite different kind, based on Mr. Tutin's quantitative data as to proportion of T.N.T. administered to rabbits which is excreted as the Webster chromogen, it will be seen that Dr. Dale, again, arrives at the conclusion that the chromogen represents probably the line of safest metabolism and excretion of T.N.T., and that its *absence* from the urine of a patient known to be absorbing T.N.T. may be the really ominous sign. It is interesting to note this convergence of the different and independent lines of clinical, pharmacological, and biochemical evidence to the same conclusion.

On a point of detail, namely, the exact chemical nature of the chromogen, a discrepancy may be detected between the statement of Dr. Wyon on the one hand, and those of Mr. Webster (its discoverer) and Dr. Dale on the other. Dr. Wyon's statement, and the view he based on it as to the significance of the chromogen in the poisoning process, seemed to be justified by the evidence available when he wrote his memorandum ; but some results already obtained by Mr. Webster, and their confirmation by the very careful work of Mr. Tutin, made it necessary to abandon this conception in favour of that which is given by Mr. Webster and by Dr. Dale.

There are several other points on which the different authors are led to similar conclusions. There is, for example, a general consensus in favour of the view that the toxic results were due to the T.N.T. (*a.* trinitrotoluene) itself, and not to any more toxic and variable impurities. On the other hand, the irregularity in the incidence of toxic jaundice, its tendency to appear in factories in outbreaks having the form of small epidemics, and the practically complete failure to reproduce comparable liver changes by the heaviest dosage in experimental animals, lead Dr. O'Donovan, Dr. Wyon, and Dr. Panton, each from his own point of view, to suggest the presence of some other, unknown factor in the production of the liver atrophy. One suggestion considered, that of an infection, harmless or trivial in its effect on the liver of the healthy man, but capable of initiating widespread degenerative changes in the liver weakened by prolonged

exposure to T.N.T., has already appeared in speculations on the causation of the outbreaks of liver atrophy, in many ways so similar, which have occurred among patients heavily dosed with Salvarsan.

The other type of fatal poisoning by T.N.T., the aplastic anaemia with which Dr. Panton's memoranda deals fully, proved more accessible to study by animal experiment. It was a much rarer accident in man than toxic jaundice, and sporadic in its distribution. No suggestion is offered for its appearance in a few cases out of many thousands exposed to T.N.T., other than individual susceptibility.

There are many questions concerning the poisonous action of T.N.T. which these reports leave unsolved. Further progress towards their solution by laboratory experiment does not at present seem probable, and with the termination of the war, opportunities for studying its effects on large numbers of human subjects have fortunately disappeared. Further light on it, if any, will probably come from the study of allied conditions produced by other substances; and, similarly, these studies on poisoning by T.N.T. may be found to have a value, independent of the pratical object with which they were undertaken, for the elucidation of pathologically similar conditions produced by a much wider class of poisons.

Medical Research Council,
15 Buckingham Street,
Strand,
London, W.C. 2.

5 November 1920.

CONTENTS

PAGE

I. T.N.T. Sickness and Toxic Jaundice, by W. J. O'Donovan, O.B.E., M.D., M.R.C.P. 7

II. Experiments on the Toxic Effects of Trinitrotoluene in Animals, by G. A. Wyon, M.D., B.Sc. 32

III. On the Metabolism and Excretion of 2 : 4 : 6-Trinitrotoluene, by T. A. Webster 49

IV. The Fate of T.N.T. in the Animal Body, by H. H. Dale, C.B.E., M.D., F.R.S. 53

V. Summary of an Experimental Investigation into T.N.T. Poisoning, by P. N. Panton, M.B., B.C., and Harold E. Bates, M.D. . . . 62

VI. Experimental Work upon T.N.T. Poisoning, and the Pathological Changes induced by T.N.T. in Human Beings, by P. N. Panton, M.B., B.C. 72

I. T.N.T. SICKNESS AND TOXIC JAUNDICE

By W. J. O'Donovan, O.B.E., M.D., M.R.C.P.

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CONTENTS

		PAGE
(1)	Historical	7
(2)	Technical	9
(3)	Clinical Effects	12
	Dermatitis	13
	Functional or Reflex Gastritis	14
	Blood Changes	14
	Aplastic Anaemia	14
	Toxic Gastritis	15
	Toxic Jaundice	15
	Symptomatology	16
	Differential Diagnosis	18
	Latent Period	22
(4)	Preventive Measures	22
	Ventilation	24
	Alternation of Employment	25
(5)	Theories of Causation of Toxic Jaundice	26
	Appendix	28

(1) Historical.

Long before the outbreak of war in August 1914, the *manufacture* of explosives involving the use of trinitrotoluene had been recognized as a dangerous process, and, as such, its use was safeguarded by inclusion in Home Office Regulations dated October 3, 1908; the substance itself, with which mines had been filled by the Explosives Loading Company, Faversham, for years, had borne a most innocent reputation as to its toxicity. Dr. Prosser White, of Wigan, a recognized authority on poisoning by the toluene derivatives, had described T.N.T. as an innocuous non-toxic substance which had never been known to produce illness. Until the end of 1915 nothing occurred to shake this prevailing belief. Toxic jaundice was added to the schedule of diseases that have to be notified to the Chief Inspector of Factories by a Home Office Order of November 1915, but this was because 70 cases of toxic jaundice with 12 deaths had occurred in various aeroplane factories from the use of tetrachlorethane, a constituent of the dope or varnish applied to aeroplane wings.

The *first death* attributed to T.N.T. occurred in the manufacture of this explosive in February 1915. A man, J. F. B., aged 34, had been employed from October 26, 1914, until February 9, 1915, as a labourer in a T.N.T. manufacturing plant. On the latter date he was suspended by the certifying surgeon for jaundice.

He fell ill on February 11 and died on February 23, 1915. Dr. Legge of the Home Office attended the post-mortem and inquest.

The post-mortem showed grey hepatization of lung in which pneumococci were demonstrated. The liver was normal in size and showed multilobular cirrhosis. It is very dubious whether this case should be properly recorded as one due to T.N.T. poisoning in which acute atrophy of the liver is the outstanding feature, and hence the following case should be considered the first authentic case of liver atrophy due to T.N.T.

On July 9, 1915, Dr. Edgar Collis, after visiting a factory in Kent, travelled on the back of a motor bicycle to Whitstable to see a patient, Rn., under the care of Dr. Rupert Smith, who had connected his patient's illness with the ' inhalation ' of trinitrotoluene, and who gave the following history :

Age 19, previously an oyster dredger ; employed filling shells and mines with trinitrotoluene for twelve weeks. Left work a month previously suffering from severe vomiting and inability to take food. He said his bowels only acted after taking salts, and that the motions were clay coloured, also that the urine was deep brown. On examination, the conjunctivae and whole surface of the body were deeply jaundiced but his general condition was not serious. Except for some depression his aspect was normal, pulse 72, lips a good colour. There was no tenderness over the liver, but Dr. Collis, on examination, thought the liver was reduced in size. The patient recovered.

The first undoubted death due to T.N.T. was a man A. W., aged 44, who worked on shell filling at Woolwich Arsenal from May 1915 to July 30, 1915, and died on August 17, 1915, in Guy's Hospital under Dr. Herbert French. The post-mortem showed the atrophic liver changes due to T.N.T., with which we have since become familiar.

In February 1916 no cases of toxic jaundice were reported, but in March, four cases, with three deaths, occurred, and Dr. Legge of the Home Office, realizing that this might become a growing menace, drew up and distributed a small book on the subject for the instruction of medical officers in the factories concerned in the handling of T.N.T. Although up to June 1916 no cases had occurred in any of the newly organized national filling factories, Dr. Legge pointed out to the Ministry the serious consequences to be expected from the handling of T.N.T. in largely increasing quantities, and Dr. W. J. O'Donovan was attached by Sir Eric Geddes, then Deputy Director of Gun Ammunition Filling, to his division, so that the work of the factory medical officers might be organized on proper lines, and the clinical and preventive experience of the different factories might be co-ordinated.

Meanwhile, the number of cases reported to the Home Office began to increase. In July of 1916 there were 16 cases and five deaths, seven of which cases, including two deaths, occurred in the recently established national filling factories. On August 2, 1916, Dr. Addison called a meeting of the representatives of all departments concerned in the use of T.N.T. and from this may be said to date the strenuous efforts, medical, engineering, and administrative, made to put a stop to this growing menace.

Before this date, precautionary measures had been considered by the Health of Munition Workers Committee, and the following

seven cardinal points were set out and may be said to have served as a basis for the elaboration of later regulations:

1. Cleanliness of premises and person.
2. Alternation of employment, by means of which workers would be alternately on and off handling T.N.T.
3. Adoption of proper costumes, particularly for women.
4. The necessity for adequate washing accommodation.
5. Adequate canteen arrangements.
6. Efficient ventilation.
7. Adequate medical supervision.

At the end of September 1916 the position of T.N.T. illness had become much more acute, the reported cases of toxic jaundice rising to 57 with 16 deaths during the months of August and September; moreover a tendency to decline work in factories where T.N.T. was being handled began to manifest itself among the workers. To cope with this latter menace, which from its effects would be so dangerous to the country, the Minister of Munitions, Mr. Montagu, appointed at the end of October 1916 an Advisory Committee under the chairmanship of Sir (then Dr.) W. M. Fletcher with Mr. Gray Barnett as secretary. This committee met almost daily for a short time. Recommendations were issued to secure clean working and good ventilation and the provision of suitable costumes. A memorandum was also issued setting out in detail the functions of factory medical officers. (Appendix p. 28.)

The existing powers of the Ministry of Munitions to enforce regulations were gone into carefully, and on December 22, 1916, Section 35 A.A. was added to the Defence of the Realm Regulations by an Order in Council which gave the Admiralty, the Army Council, and the Ministry of Munitions powers to enforce in any factory any regulation considered necessary in the interests of health.

On January 6, 1917, draft regulations prepared by a sub-committee were finally approved by the T.N.T. Advisory Committee and submitted to the Minister, Dr. Addison, on their behalf by the chairman with an explanatory letter of January 10, 1917. These rules in an amended form were finally issued on March 30, 1917, with a covering memorandum originally drawn up by the T.N.T. Committee.

Feeling that the main work of the T.N.T. Committee had ceased with the issue of these regulations, the Minister of Munitions, now Mr. Churchill, decided that the functions of the T.N.T. Committee lay now in the field of scientific advice, and a T.N.T. Advisory Committee, consisting of Sir Walter Fletcher, Dr. Collis, Dr. Dale, Dr. Legge, Dr. Moore, Dr. O'Donovan, and Dr. Panton, was constituted for the initiation or direction of further research work and its application to the practical problems of the Ministry.

(2) Technical.

An explosive is a substance which on detonation liberates a large volume of gas with great rapidity. The two factors of power and violence are both essential. It is well recognized that petrol in burning gives off more power than is liberated by the detonation of a high

explosive in the ratio of something like 10 to 1. But burning petrol is a comparatively slow process and hence the effects of the burning of petrol and the detonation of an explosive can hardly be compared. The power of an explosive is commonly decided by making a hole in a lead block, plugging the hole with a steel bung, and then firing the explosive by means of a fuse. Under these conditions the lead block will swell out and water poured into the cavity will give a rough indication of the amount of gas liberated. The violence of an explosive is measured by the rate at which the explosion spreads in a column of material, technically spoken of as the velocity of detonation. The power of T.N.T. is about 95 per cent. of that of picric acid. The velocity of its detonation is about 7,000 metres a second, that is to say a wave of detonation will run through a column of T.N.T. at 7,000 metres a second as compared with 7,300 for picric acid.

T.N.T. is relatively a scarce commodity. Ammonium nitrate can be manufactured in very large quantities, and therefore to economize T.N.T. and to make the fullest possible use of ammonium nitrate, dilution of T.N.T. by ammonium nitrate was a matter of early trial and experiment. The earliest mixture was roughly half of T.N.T. and half of ammonium nitrate. This was sufficiently fluid when hot to be poured under favourable conditions, but it was found to be a little too thick for factory operations, so that gradually the mixture became one of 40 per cent. ammonium nitrate and 60 per cent. of T.N.T. or 40/60 AMATOL, but in the latter months of the war another mixture was introduced consisting of four parts of ammonium nitrate to one part of T.N.T., 80/20 amatol. As regards power, amatol is more efficient than either picric acid or T.N.T. alone; taking picric acid as a standard and calling it 100, the power of 40/60 amatol is 116 and 80/20 amatol is 127, and as regards crater effects an explosive containing a large proportion of ammonium nitrate proved better than an explosive consisting of pure T.N.T.

A great merit, however, of a high explosive is its insensitiveness. Amatol can be filled into a shell in a manner that would be dangerous with black powder: the firing of a charge of cordite behind a shell is sufficient to give its contents a severe shock, and if the shell were filled with a sensitive explosive there would be a very real risk that the shock of the charge would cause immediate detonation of the shell contents. In firing, too, against concrete dug-outs or armoured shelters it is important that the shell shall not detonate upon impact but shall at first attain some measure of penetration. Experience has shown that shell cases have been knocked in by the detonation of other shells and yet the amatol contents inside have not been detonated. The importance of this factor to ammunition dumps cannot of course be over-rated.

The manner in which insensitive explosives are detonated is by means of a series of relays. The detonation is started by mercury fulminate which is very sensitive and fairly violent. This sets off an intermittent explosive called tetryl. The third relay or stage is crystals of T.N.T. Between the crystals of T.N.T. and the amatol in the shell is some cast T.N.T.



This illustration shows the hand-stemming of T.N.T. into a shell.

A girl in working costume on the left is weighing a charge of amatol which is emptied from the scale-pan into a metal receiver. The man on the right is emptying a charge into a shell and the workmen in the middle is hammering down the powder with a wooden rod and mallet. The exposure of the worker's hands and soiling of the floor is evident.

It will be seen from these remarks that T.N.T. may be used as an intermediate factor in the detonation of an amatol-filled shell or it may be used as amatol for the filling of grenades, howitzer bombs, and shells of all sizes. These latter terms call for no explanation.

Exploder Bags.

These consisted of cylindrical batiste bags holding about 19 drs. weight of T.N.T. They were originally filled by hand through funnels, then by horizontal or vertical machines in the nature of sausage fillers with automatic arrangements for starting and stopping when the required weight was put into the bags. After filling the bags were tied with silk thread and the superfluous silk and batiste were cut away.

In the latest form a paper carton replaced the batiste bag, the open end of which was closed after filling by a cardboard disk. This method could be worked to a greater degree of accuracy than was possible with the batiste bags; it reduced the amount of man-handling of T.N.T., and the cost of filling these cartons was considerably less than the filling of batiste bags by machinery. Most recently the T.N.T. was filled by automatic machinery directly into the metal exploder container without any bag or carton at all. This most rational way was the last thought of. All filled shells had a cavity left at their summit into which the exploder container was screwed, and the direct filling of the charge into this cavity was a noteworthy explosive and medical improvement in the handling of T.N.T.

Methods of Shell Filling.

The early method of filling molten amatol through spigots into shells involved the labour of scores of workers removing caked amatol spilt on to the outside of shells and on to trucks, floors, and runways. In one large factory, on each of two shifts in the melt house there were between 50 and 60 women workers almost wholly employed in cleaning up owing to the spilling of amatol. Many workers engaged in trucking and other work, apart from the actual filling of the shell, came into contact with T.N.T. Dr. Moore noted that the heaviest incidence of both fatal and non-fatal cases of jaundice was found at one factory among the cleaners and truckers and not among the melters and pourers.

In filling shells by the press process, T.N.T. and ammonium nitrate had to be separately melted and then mixed. The amatol mixture was conveyed by trucks or automatic conveyors to press houses where the correct charges were weighed by hand and emptied through shell funnels into the waiting shells. These charges were rammed home by means of a process of tamping or stemming; the stemmer poured the powder through a funnel inserted into the shell opening and then hammered down the powder by striking the upper end of a wooden rod with a wooden mallet. At each stroke a fine gust of powder was driven up between the stem and the side of the funnel and another gust between the funnel and the side of the shell. In consequence of this a thick layer of dust fell upon the trucks,

floors, and workers, and so was inevitably distributed out of the precincts of the press house along the runways and into the shell stores.

Screw Filling.

For labour saving reasons in June 1916 technical officers of the Ministry were interesting themselves in mechanical methods of filling exploder bags and shells, but real and speedy progress was made when the need for eliminating hand work for medical reasons was put before the responsible officers of the Ministry of Munitions ; on December 18, 1916, at a meeting of factory managers at Aintree a demonstration of a filling machine in action was given.

Lord Chetwynd first took up the question of shell filling at Chilwell in September of 1916. His initial experiments were carried out with a corkscrew revolving inside a metal tube passed through the neck of a glass bottle and in March of 1917 an extremely satisfactory vertical filling machine with a rising hopper was in use at this factory. But before this date toxic jaundice at this factory had become an almost negligible factor. With this machine 6-inch shells could be filled in 45 seconds. A horizontal machine was the latest type brought into use, superior in some ways to the vertical machine since manipulation is easier in the horizontal position, and on the withdrawal of the filling screw any T.N.T. dropping from the end of the screw falls clear of the shell into a tray below and hence contamination does not spread away from the actual site of filling.

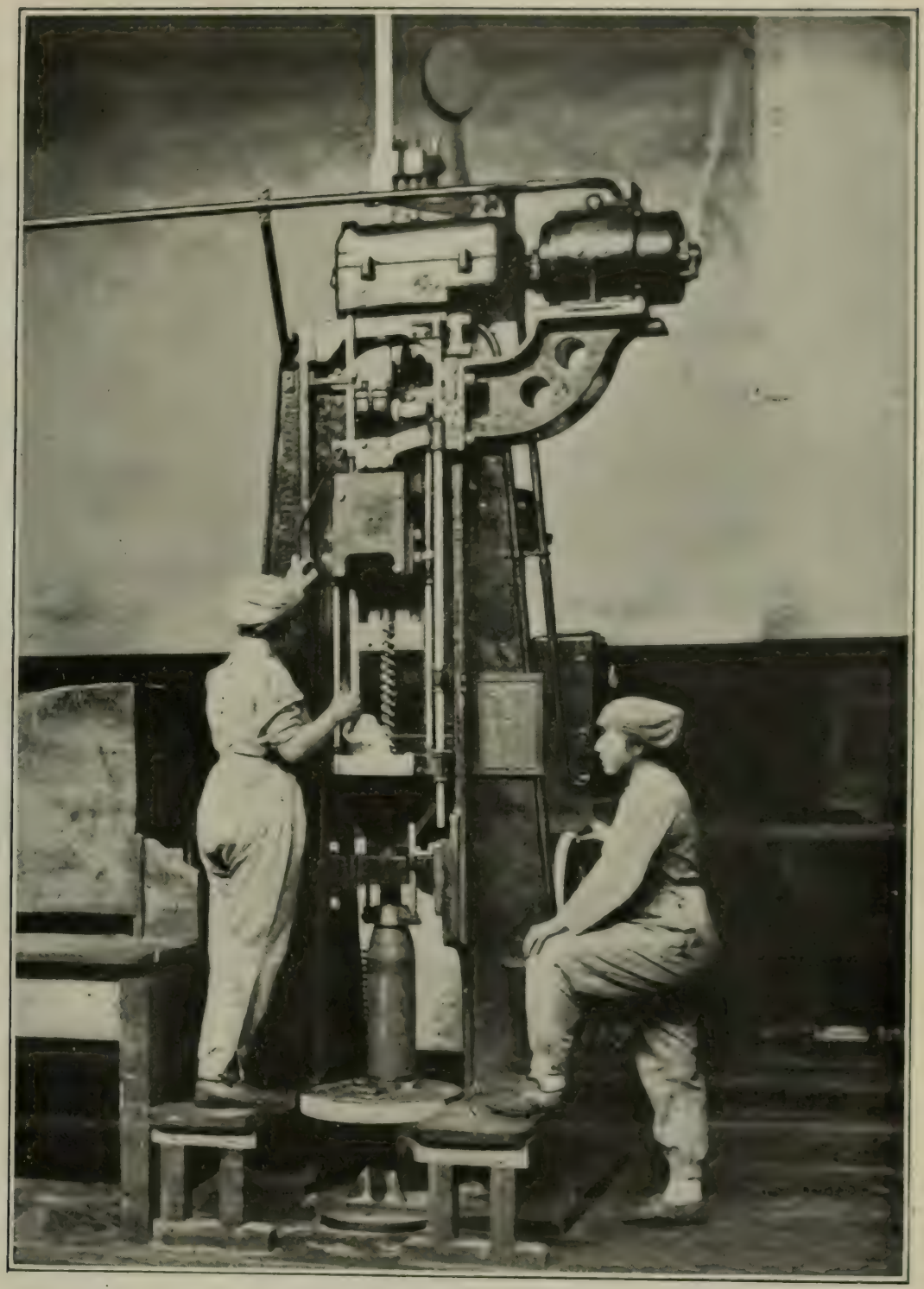
Hand Grenades.

Hand grenades of such patterns as the Mills grenade and the 'Egg' grenade owing to their internal structure were of necessity filled by hand. The actual process consisted of filling a metal cone about 18 inches long with an explosive mixture. Compressed air was then admitted to the base of the cone and its apex was inserted into a filling hole in the side of the grenade. A dozen bangs of the grenade on the work bench distributed the powder evenly in the grenade. In this process the work bench and grenades were thickly dusted with ammonal and deep staining of the workers' hands was common, yet although many thousands of these grenades were filled by hand, dermatitis has been the only ill effect noticed in the process of grenade filling. A partial explanation of this paradox of dirty work and good health can perhaps be found in the low percentage of T.N.T. present in the filling mixtures used, viz. 10 per cent. or less.

(3) Clinical Effects.

The ill effects that T.N.T. workers suffer from can be classified under five main headings :

1. Dermatitis.
2. An early, probably reflex vomiting.
3. Affections of the blood or blood-forming organs.
4. Toxic gastritis.
5. Toxic jaundice.



Chilwell stemming machine in operation ; powder being fed to hopper by the girl on the platform.

(Although by analogy with other benzine derivatives one would have expected cases of peripheral neuritis and of toxic amblyopia among T.N.T. workers no such cases have come before the medical officers of the Ministry.)

Dermatitis.

In this susceptibility may be said to have played a large part. Most workers were never attacked, whereas a few suffered severely from it even after exposure of slight duration. Most frequently it was met with in the first week of exposure, but very intractable cases were met with in workers who had handled T.N.T. with impunity up to twelve months. The parts most frequently affected were the wrists and ankles and neck, where rubbing with contaminated clothing occurred, but facial and intercrural attacks were not uncommon. The rash usually appeared as a superficial, follicular or widespread erythema, but on the hands a chronic eczema or cheiropompholyx type of eruption were also very common. Generally, T.N.T. skin affections yielded readily to simple treatment, but septic secondary infection and thrombo-phlebitis were occasionally seen. In all forms itching was often intolerable. Yellow staining of the hands and occasionally of the feet of T.N.T. workers was universal. The staining was a deep orange colour produced after very slight contact in those having greasy skins, and was most marked among operatives handling T.N.T. containing a high percentage of impurities. There was no connexion between the prevalence of dermatitis and the amount of T.N.T. sickness, and only in a very few cases have the same patients suffered from dermatitis and constitutional ill effects either together or at subsequent periods.

Powder Holes. The occurrence of ulcers in the skin and mucous membranes due to industrial work has been recognized for some time among chrome workers, workers in fulminate of mercury and other dusty occupations, but it was not until August 1918 that the occurrence of powder holes was observed among T.N.T. workers.

On August 19, I (W. J. O'D.) visited Messrs. Curtis's & Harvey's factory at Faversham, and the T.N.T. workers on that date, in all 49, were carefully examined. Twenty-six of these showed minute ulcers on the fingers attributable in the first place to injury. The fingers showed recent cuts, recent blisters or dried old blisters, some of which were black with hardened blood clots. It was noteworthy that these injuries had not healed in the usual clean fashion customary in engineering and other workshops, but the tendency was for these linear cuts or abrasions of the fingers and thumbs to become shallow round ulcers which at the time of my visit were of four, five, and up to eight days' duration. Of even greater interest was the presence of five cases showing definite ' powder holes ' in the webs between the fingers or between the fingers and thumb. Noteworthy features at the visit were the free sweating and deep walnut staining of the workers' hands, and the tremor of the hands when extended. The tremor was attributable to the effects of a recent explosion and was less marked than it had been some days previously. It was striking that none of the workers handling flaked T.N.T. as opposed to those handling amatol powder showed any of these cutaneous

effects, nor did the girls whose work consisted of dipping solid pellets of T.N.T. into molten wax show any such lesions: hence, the hygroscopic property of ammonium nitrate, the major constituent of amatol, must be at least an important factor in the production of the powder holes. A striking feature was the absence of complaint by the workers as to any suffering from these cutaneous lesions; but such anaesthesia is common with powder holes due to industrial processes, and is the reason why they often go untreated for weeks, and on this account perforations have been recorded that reach down to the phalangeal bones.

Such 'powder holes' are to be attributed to the continued attrition of caked amatol and sweat stuck in rolls in the webs of the fingers, and the non-healing and ulceration of small cuts and abrasions is to be put down to the irritant effect of amatol upon undressed cuts.

Functional or Reflex Gastritis.

Among new workers vomiting in the early morning and occasionally after food during the first fortnight was a fairly frequent complaint; absentees from this cause were extremely rare. Similar complaints were made among workers in fulminate of mercury and in tetryl sheds, and the issue of free milk and a purge was in all cases sufficient to bring about a cure. The explanation of this seems to be largely psychic, inasmuch as after the first two years hardly any sickness of this type was seen by the factory medical officers.

Blood Changes.

Noticeable pallor of T.N.T. workers in the early days led to a special investigation of this question by Dr. P. N. Panton. The effect of these researches was to narrow down the field of blood changes to a uniformly fatal lesion termed *aplastic anaemia*. In all 15 cases of aplastic anaemia are known to have occurred and all are now dead. Four only had previous toxic jaundice.

Aplastic Anaemia.

This disease may show itself as gradually increasing debility and breathlessness as found in pernicious anaemia, or the onset may be sudden and haemorrhagic. The haemorrhage may show itself first on the legs, as a general purpura, or as a case of severe uterine bleeding. Too much stress cannot be laid on the necessity for a skilfully conducted post-mortem inasmuch as a fatal uterine haemorrhage has certainly in two cases been ascribed wrongly to abortion and the cutaneous haemorrhages of both septicaemia and leukaemia may be and have been wrongly ascribed to T.N.T. when occurring in T.N.T. workers.

There is no parallelism in the incidence of toxic jaundice and aplastic anaemia. One factory, 'C', with the largest number of cases of toxic jaundice, has had only one case of aplastic anaemia, whereas another factory, 'H', with only a few cases of toxic jaundice, has had four cases of fatal anaemia.

Toxic Gastritis.

This was the outstanding ill-effect experienced in all filling factories. The incidence varied from factory to factory, but appended is the incidence of sickness in the factory which suffered more than any from this disease. The number employed averaged about 6,000 throughout the period. The amount of sickness in percentages on the different processes was as follows :

Week ending. 1916.	*Melt filling.* Male.	Female.	*Press filling.* Male.	Female.	*Incorporating mills.* Males only.
October 7 . .	4·0	5·9	3·2	2·5	5·3
October 14 . .	7·7	10·0	4·3	5·1	5·7
October 21 . .	11·4	8·7	3·8	7·2	7·4
October 28 . .	4·1	3·1	4·5	5·6	3·0

Pain was the general and often the only complaint. This pain started in the epigastrium or in the lower chest, always local, and not radiating into the back or in any other segmental area, it varied from tolerable discomfort to great severity, was colicky in character, and had no definite relation to meals. Rest relieved the pain. Neither food nor starvation were palliatives. Vomiting rarely coincided with this pain and when it occurred gave no relief. Nausea and aversion to food were concomitant symptoms, and loss of weight went hand in hand with this. Constipation was the rule ; the presence of diarrhoea always made one suspect the case was one of food poisoning rather than a real effect of T.N.T. absorption. On examination these patients showed marked apathy and muscular weakness, their faces were pallid, drawn, and wizened, their sclerotics were dulled, their tongues clean. Highly coloured urine and frequency of micturition were commonly complained of by these patients. In these cases cyanosis of lips and tongue was generally notable. Abdominal examination in a small proportion of cases showed epigastric tenderness, but usually nothing. These symptoms would come on at any time from a fortnight to nine or ten months after employment on T.N.T., and might exist for a week or two before medical help was sought. Two to six weeks might elapse before the patient was again fit for work. Open air and purgatives were proved the best form of treatment. Relapses were not common, but when they did occur were a warning that this particular patient should be kept from any further contact with T.N.T. A noticeable feature among these patients was the undue prolongation of convalescence owing to the fear of return to work at shell filling, and from this cause chronic invalidism at one time loomed heavily in the statistics of factory illness.

Toxic Jaundice.

Up to the last the factory doctors maintained the view that toxic jaundice might appear without symptoms, and the fact that there had to be an elaborate organization for picking these icteric cases out from among their fellow employees while at their work is sufficient evidence that the early onset of jaundice need not be associated with sufficient symptoms to lead the patient to seek medical advice.

It was an early experience that young adults were frequently attacked and when attacked experienced a high mortality. In consequence of this, employment in T.N.T. work below the age of 18 was prohibited. It has been suggested at different times that alcohol, syphilis, adenoids, obesity, and bad feeding, are predisposing causes, but no evidence is available pointing in any of these directions. The youngest case of toxic jaundice reported was a boy aged 14, the eldest a man aged 52.

The *duration of employment* on T.N.T. before the onset of jaundice varied from three days to seventeen months : the greatest incidence of jaundice fell in the third month of employment, but one has to remember in this connexion the instability of the women workers in the filling factories ; the subjoined table by Captain M. Greenwood, 18.3.18, shows very clearly the constitution of a munitions population by length of service.

Length of service.	*Number (percentage).*
1 month	13·33
2 months	9·66
3 „	10·00
4 „	11·61
5 „	10·04
6 „	7·46
7 „	5·89
8 „	5·67
9 „	5·87
10 „	4·06
11 „	5·59
12 „ and over	10·81

Symptomatology of Toxic Jaundice.

The early signs and symptoms of toxic jaundice were of paramount importance, since the earlier these cases were removed from their work the better was the prognosis.

Of 30 cases experienced by one medical officer, 15 had premonitions, their priority varying from a day to a month, generally about a week, of dizziness, tiredness, headaches, and an overpowering desire to sleep in the daytime. Dyspepsia, abdominal pain, and frequent vomiting, were present in varying degrees in most of these cases. In the remaining 15 cases, however, *there was no warning.*

The earliest published observations by Dr. A. Livingstone-Learmouth and Dr. Barbara N. Cunningham stated, in the *Lancet* of August 12, 1916 :

> ' It is curious that there seems to be, as it were, no constant relationship between the severity of the symptoms complained of and the ultimate issue of the case. A worker with severe gastric derangement when taken off T.N.T. may completely recover in a short time, whereas slight loss of appetite and lassitude may (although the worker is removed from all contact with the powder) prove the gateway to serious illness and physical disaster. A patient, too, may be jaundiced for some time, and present no more symptoms than are seen in a simple catarrhal jaundice until a week or so from the end, so far as our limited experience of the serious cases enables us to judge.'

Workers deeply jaundiced, such as were met with in the early days of intensive output, were picked out by the medical officers, and these workers often resented strongly any interference with their continuous employment.

In many cases an absolute diagnosis of jaundice was difficult in its early stages; so many workers have a transient loss of the whiteness of their sclerotics from ill-health, constipation, and other causes, and here a laboratory test has been used with great advantage. A little blood of a suspected worker is drawn into a collecting tube and allowed to coagulate. The serum above the clot is occasionally a vivid yellow colour, and it was the practice of Dr. Castellain at three large filling factories in succession to remove from T.N.T. contact any one whose serum gave such a positive colour test. The factory nurses early became adepts at performing this little routine investigation, and only positive cases were brought to the notice of the factory medical officers.

Vomiting. This symptom in cases of toxic jaundice was often severe, recurring with each attempt to take solid food; it was rarely absent when jaundice was at all severe. The appetite was generally very good, except when patients were prostrated in a cholaemic state. Constipation was the common accompaniment and the stools were generally white and hard, though natural coloured stools were passed in some cases and viscid bile in fair quantity was often found in the gall bladder at post-mortem examinations.

Abdominal Pain when present was in the liver region and marked tenderness on pressure over the liver area was occasionally elicited.

Liver Dullness. Although this is a somewhat variable area depending on the obesity of the worker, the degree of emphysema and flatulence existing in a particular case, nevertheless many definite observations of diminution of liver dullness have been made by different practitioners and a complete absence of liver dullness before death has been twice noted. In early and slight cases a slight increase of the liver dullness has been noted, but it is impossible to say that in such slight non-fatal cases there were present in fact the typical T.N.T. atrophic changes. Dr. Barbara Crawford has reported a case in which in five weeks the liver dullness from being less than one inch increased to two and a half inches (*B.M.J.*, April 20, 1918).

This regeneration after extensive reduction is indeed noteworthy.

In two cases of toxic jaundice X-rayed, one by Dr. Finzi and one by Dr. Gilbert Scott, shrinkage of liver was demonstrable on the screen.

Ascites. Although a slight amount of ascitic fluid is not uncommon at the post-mortem of these cases, its presence in any quantity sufficient to be demonstrable clinically has been very rare.

Fatal Case of Ascites.

F. C. W., aged 48. Measles, yes; scarlet fever, no; rheumatic fever, no; pneumonia, no. Never abroad. Labouring work usually. Went to Barnbow N.F.F., July 23, 1918. Passed in as fit. Worked on pressing, machine stemming (80 20 cold), and hot mix (80 20) machine stemming. No alternation of employment.

Well until October 3, 1918. Slight jaundice noticed. Complained then to doctor of dragging in lower chest and upper abdomen which is getting worse.

On examination (October 16, 1918). No vomiting. Slight headache. No delusions. Stools grey. Urine reddish. Sweat not noticed. Appetite gone. Bowels loose. Pruritus troublesome. Feels weak and tired. Intense yellow

sclerotics and skin. Heart rhythm, size, and sounds, natural. Pulse 84, regular. Lungs crepitations at both bases behind. Abdomen distended, flat umbilicus, *shifting* dullness, fluid thrill; oedema of sacrum. Slight oedema of ankles.

Post-mortem by Capt. M. J. Stewart (Leeds Royal Infirmary). Peritoneum contains several pints of clear, deeply bile-stained fluid. Liver shows considerable reduction in size. Weight 39 oz. The surface of the organ presents the typical appearance of T.N.T. atrophy, &c. . . . The larger bile ducts and the vessels of the liver present no naked eye change. The gall bladder contains yellow normal looking bile and its mucous membrane appears normal.

Pulse Rate. The pulse rate varies even in similar cases. Of three cases seen on the same day in Hereford Hospital, October 29, 1918, F. B., severe, pulse 100–60; M. H., slight, pulse 44; and B. E., severe, pulse 42–76.

Pruritus. This symptom may be present in jaundice due to T.N.T. atrophy as in other cases of jaundice, it was often entirely absent.

Pyrexia. As a rule patients with toxic jaundice show a normal temperature chart. An irregular pyrexia of 102° Fah. is not uncommon. High temperature by no means indicates a fatal issue.

Case of T.N.T. Liver Atrophy with High Fever, recovery. (Chart A).

F. T., aged 19, single. Temp. 104·4, recovery. After one month's filling shells with molten T.N.T. the patient felt unwell with pain in the stomach, vomiting, slight diarrhoea, and jaundice. Admitted to the Nottingham General Hospital October 16, 1916. Abdomen; no rigidity, no tenderness, no enlargement of organs. Urine 28 oz. per diem, bile present.

The succeeding temperatures were normal or subnormal until the discharge, 27.11.16.

Case of T.N.T. Liver Atrophy, Delirium, and slight Pyrexia, recovery. (Chart B).

T. M., male, aged 22. Worked in T.N.T. from January 25 to April 5, 1917, 5.4.17 complained of faintness: 16.5.17 saw Dr. C., epigastric pain, conjunctival and general icterus noted, admitted to hospital: 2.6.17 delirious, incontinence of urine and faeces, greatly emaciated. Liver dullness 1½ inches in nipple line: 22.6.17 liver dullness barely 1 inch: 27.7.17 discharged, no symptoms, no jaundice, liver dullness 2½ inches: 6.8.17 occasional pain over liver: 27.8.17 liver dullness normal, discharged from factory at own request to take up work in a pit.

Mental Symptoms. In cases of established jaundice the mentality of the patient is usually unclouded and hopeful and patients in bed in hospital usually express a desire to eat more than is given them and to be up and about. This optimism has been aptly likened by Dr. J. A. P. Barnes to *spes phthisica.*

This want of comprehension of the gravity of their illness has been compared by many to that found in cases of puerperal septicaemia, and has made the retention of these patients in hospital a matter of difficulty. Unconsciousness or a quiet delirium may supervene quite suddenly in patients jaundiced and feeling well, but such cases may recover.

Differential Diagnosis of Toxic Jaundice.

The diagnosis of toxic jaundice has always been a matter of difficulty that increased with the experience of factory medical officers.

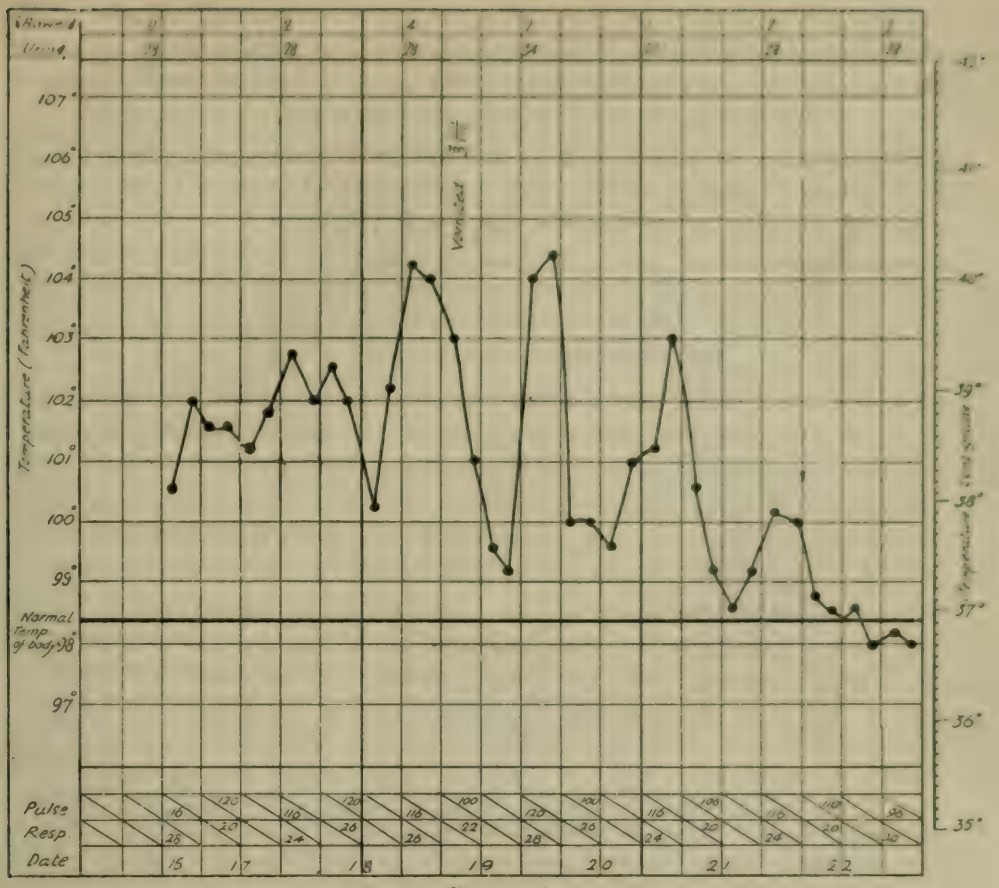


CHART A

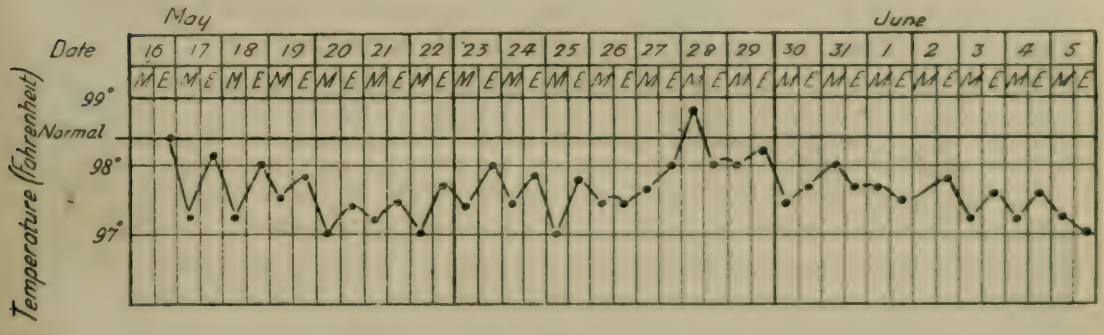


CHART B

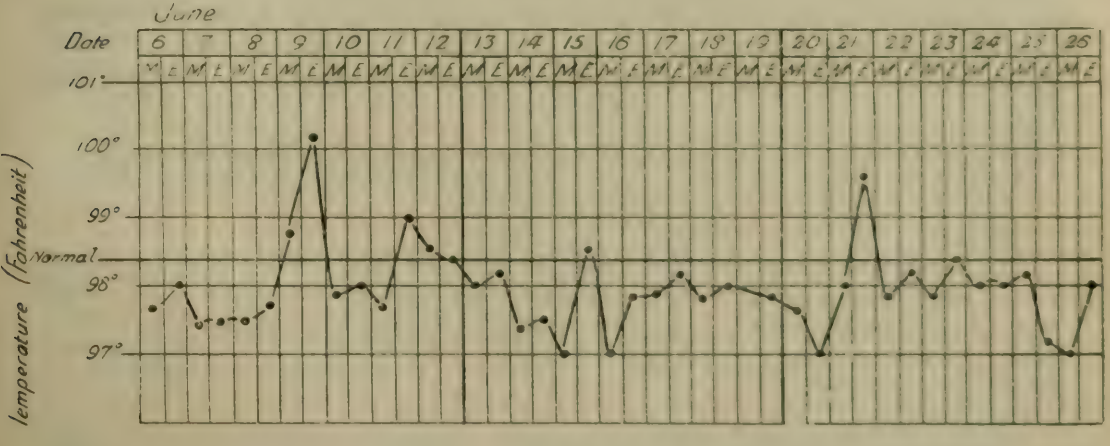


CHART B (*continued*)

B 2

When cases are picked out with slight icterus from among the general body of the workers these cases are symptomless and apyrexial and the factory doctors uniformly stated their inability to distinguish these cases from the catarrhal jaundice of ordinary experience.

This difficulty of diagnosis has been previously referred to by Dr. H. Morley Fletcher of St. Bartholomew's Hospital (*Proceedings of the Royal Society of Medicine*, January 1917).

In severe cases of rapidly increasing jaundice, generally the diagnosis of liver atrophy will be correct, and the exclusion of the rarer causes of grave jaundice among young and middle aged munition workers is not as a rule difficult.

Cases of secondary carcinoma of the liver, familial acholuric jaundice, of gall stones, and large cirrhotic livers, have been met with; but their exclusion, as being cases not due to T.N.T., is not difficult after full investigation.

A noteworthy feature in cases of toxic jaundice has been the absence of Webster's reaction in the urine of these patients, and Dr. Barbara Crawford carried out the following experiment to see whether or no the inability to excrete the T.N.T. derivative giving Webster's reaction might be used with safety for diagnostic purposes:

'From a series of several hundred examinations of the urine my experience has been that T.N.T. is normally present (Webster's test) when a worker is in contact with this substance, and that instead of this being a danger signal, as has been held, it is on the contrary normal and quite compatible with good health; it is not found in the urine of patients suffering from toxic jaundice. I have recently confirmed this at the suggestion of Dr. O'Donovan, by rubbing a small quantity of an ointment containing 12½ per cent. of T.N.T. into the skin of a healthy adult as control. The urines were tested for the next 48 hours with the result that no T.N.T. was detected in the toxic jaundice patient's urine, while it appeared in that of the control after a few hours.' April 1918.

A diminution in liver dullness that progresses while under observation might be regarded as definitely diagnostic in a T.N.T. worker of toxic jaundice.

A diagnosis after the event in these cases is often difficult. A patient who has given a history of jaundice after work on T.N.T. associated with prostration, white stools, and pain in the liver area, may later show a fairly clear picture of cholelithiasis. It is to be remembered, however, that gall stones have been found post-mortem in cases of T.N.T. workers dying of liver atrophy.

It may be held that the presence of gall stones may predispose a worker to an attack of toxic jaundice, and the present certain diagnosis of gall stones does not negative a previous jaundice of toxic origin.

Illustrative Case of Gall Stones and Jaundice in T.N.T. Worker.

Mrs. L. Under Dr. Alexander of Faversham.

Married. One child. Husband alive and well.

First went to work at a factory in Faversham, May 1917. Was employed as an examiner gauging T.N.T. pellets on alternative fortnights. This work stained her hands yellow. Alternate fortnights were spent on non-T.N.T. work. She last left the factory about beginning of November 1917. She was jaundiced about November 23, 1917.

Onset of Illness:

Symptoms: Headache, nausea, malaise. Attacks of pain in R. hypochrondium, which came on at any time day or night, severe, radiated up to right

shoulder and bore no relation to meals. She shivered and sweated in attacks of pain. No vomiting. Her friends noticed her jaundice concurrently with onset of symptoms about a week before she saw a doctor. She has improved since Xmas 1917, though when up a fortnight ago her feet swelled. She still has some pain in R. hypochrondium.

On Examination :

A well built, well covered, fresh complexioned brunette. No cyanosis. No rash. No stains on skin. Tongue clean. Glands not enlarged. Heart : natural rhythm, rate and sounds. Lungs resonant all over, good air entry. Abdomen not distended, no ascites, moves well. A band of hyperesthesia (10th dorsal segment) on right side of trunk from vertebral column to umbilicus. Liver not enlarged, not diminished to percussion, not tender on pressure. Gall bladder : a tumour about an inch across palpable in G.B. area, descends with inspiration, very tender on pressure. Urine has contained bile off and on since first attack.

Diagnosis (11.2.18.) :

Gall stones with attacks of colic and some inflammation of gall bladder. The original diagnosis of Toxic Jaundice in 1917 is open to question.

Fatal Case of T.N.T. Liver Atrophy with Single Cholesterin Gall stone.

Case of J. S., aged 49. *Morecambe.*

Worked in T.N.T. from January 1917 to July 13, 1917.

It is uncertain whether he was medically examined before commencing, all records having been destroyed in an explosion.

July 13, 1917. Noticed to be slightly jaundiced and sent to Lancaster Infirmary.

July 29, 1917. House surgeon could not detect any jaundice.

August 11, 1917. Discharged from infirmary.

October 16, 1917. Death.

Post-mortem, October 17, 1917. Liver 34 oz. Many adhesions, subacute yellow atrophy.

Oval cholesterin gall stone (4×2·5 cm.) present in gall bladder, duct impermeable.

The gall stone was very light in weight and almost floated in water. The surface was smooth and light green. On the cut surface was a peripheral zone (0·15 cm. broad) of soft friable cholesterin tinged by bilirubin. Internal to this were radially arranged large crystals of cholesterin : these were clear and glassy except in the centre of the stone where they were rusty.

Fatal Case of T.N.T. Liver Atrophy with Twelve Gall Stones

M. J., aged 22. Single. Morecambe National Filling Factory.

Commenced work January 17, 1917. Stemming eight weeks. At the end of this complained of headache, vomiting, and abdominal pain. Transferred to kitchen cleaning on February 26, 1917.

March 16, 1917, reported and asked to be sent back to powder. Conjunctivae noticed yellow. Kept under observation till March 20, then sent to Lancaster Infirmary, general jaundice.

Died April 27, 1917.

Post-mortem May 2, 1917. No skin petechiae. Liver 23 oz. No recognizable liver pattern, close set grey points of fibrous tissue. Right lobe contains an area 3″×4′ of coalesced pin head yellow nodules. Gall bladder, old adhesions to liver and duodenum. No thickening of wall, contained 12 facetted stones. Bile duct patent.

Fatal Case of T.N.T. and Liver Atrophy associated with Hydatid Cyst.

A. F., aged 22, single, Morecambe National Filling Factory.

Previously a weaver.

Medically examined and passed fit February 13, 1917. Stemming February 13–27, 1917. Transit shed till March 6. Stemming and trucking T.N.T. till March 9.

Left factory April 2, 1917, and returned to home in Burnley ; was noticed to

be yellow on arrival. She did two days' weaving but became breathless and saw Dr. Scott, who certified T.N.T. poisoning.

Admitted Burnley Infirmary April 28, 1917. Collapsed, persistent vomiting, general jaundice, delirious, no temperature.

Death May 6, 1917. *Post-mortem.* Blue subcutaneous haemorrhage left shin. General icterus. Liver 38 oz. Left lobe very small tongue. On section red tissue without recognizable liver pattern. Discrete or coalesced yellow lobules scattered throughout. On convexity of right lobe and on under surface of right lobe internal to gall bladder are two hydatid cysts, 3 and 4 inches in diameter. Severe fatty degeneration of heart and kidneys.

Latent Period.

A remarkable feature of both toxic jaundice and aplastic anaemia in a few cases has been the latency of the condition, that is, the length of period that elapsed between absorption of the poison and the development of jaundice or of anaemia.

The first noted case of this latency was a boy C. N., aged 16, who worked in a melt house from May 23 to the end of June 1916. He was discharged for threatening to blow the place up. He worked at his father's farm from the end of June to August 28 and in the middle of this period felt sleepy for three days. On August 28 he saw his doctor for vomiting. On September 1 he was jaundiced and on September 9 he died. A post-mortem two days later showed an 18½ oz. liver with typical changes of acute atrophy and fatty degeneration.

Another case reported by Dr. Legge in the annual report of the Chief Inspector of Factories and Workshops for the year 1917 was a woman who after two months' work in the manufacture of T.N.T. was transferred to another department where she was employed in filling drums with benzol and toluol. Over seven months later she met with an accident, a spanner falling on her head. Toxic jaundice developed one week later and proved fatal in a fortnight.

The longest period of latency known has been published by Professor Glynn where the latent period extended over nine months. The patient was a female aged 20, single, and was employed from January 1917 up to September 1917 in a filling room where eighteen-pounder shells were filled with amatol and T.N.T. blocks. Her duty was to handle the filled shells. During this period her health was good. She remained well until June 5 when she vomited. On June 7 slight jaundice appeared and she died in the Liverpool Royal Infirmary comatose on June 14. *Post-mortem.* Liver weighed 41 oz. Capsule not wrinkled. Surface, yellow ochre, mottled with small sub-capsular haemorrhages; on section very soft and uniformly yellow. Microscopical examination of the liver showed extensive focal necroses affecting about a third of each liver lobule (central portion).

(4) Preventive Measures.

The preventive measures that have been in use were direct and indirect in their action.

Under indirect measures are included the provision of canteens, good meals, the shortening of hours, the facilitating of workers' transit to and from work and the introduction where possible of

breaks to lessen the periods of continual exposure. Of indirect benefit, too, was the installation of factory dentists and the preliminary medical examination which weeded out the more unfit, especially those with dyspeptic or bilious derangements, from entering on a T.N.T. occupation.

Direct measures consisted of steps taken to eliminate contact between the worker and the explosive. These steps included the provision of ample washing accommodation and the supervision of its use, the provision of protective clothing and caps, in some cases provision of factory underclothes to be worn while at work, and the installation of compulsory bathing.

The presence of a resident medical officer in the workshops of the factory served two purposes : (1) to give an eye to the maintenance of cleanly conditions of working, and (2) to pick out those showing signs of T.N.T. absorption and cause their removal from this work before absorption had proceeded to a dangerous degree.

A great elimination of possibilities of contact occurred when steps were taken in the end of 1916 to secure that floors, vessels, and trucks were constantly cleaned, but the multiplication and improvement of mechanical methods of filling was without doubt the biggest factor in the elimination of contact.

The absolute elimination of contact under factory conditions is hardly conceivable, for wherever T.N.T. is handled in bulk there will be a slight and perhaps invisible film of T.N.T. dust on all exposed surfaces. No one who remembers the conditions of working in the summer of 1916 will question that early in 1917 a great change was observed in factory conditions, and by the end of 1917 mechanical working had reached a high state of efficiency. Mechanical arrangements of all sorts had to be thought out and the machines made. This necessarily took time while output could not be delayed.

In any discussion on protective clothing reference must be made to gloves. Their uselessness was referred to by Simeon Snell in 1894 (*B.M.J.*, March 3) and when one realizes the time and care taken to inculcate into medical students an aseptic habit in the donning of gloves it is easy to see why we completely failed to teach workers how to wear gloves in a safe fashion. Experience showed that after a few hours' work the inside of the glove became contaminated with T.N.T. and then the worker's state was potentially worse than had gloves not been worn. Dr. Benjamin Moore reported that he once succeeded in getting the hands chemically clean of one girl by asking her to go to the laboratory before going on and after coming off each working period and by putting on and taking off the protective clothing himself.

Gloves were provided and worn by the million, and in the end their use was universally discarded, although for hot work or to protect the girls' hands from sharp edges of steel their use had to be tolerated.

Clean working necessarily connotes skilled working. The chart (C, p. 24) made by Dr. Castellain at Hereford shows a close parallel between the incidence of T.N.T. sickness and the incidence of accidents.

Between August 3 and August 17, 1918, owing to a great and destructive explosion at Chilwell, the workers at Hereford were

increased by over a thousand. This meant a great leavening by unskilled and untrained workers, whose sickness rate and accident rate showed an immediate and heavy rise.

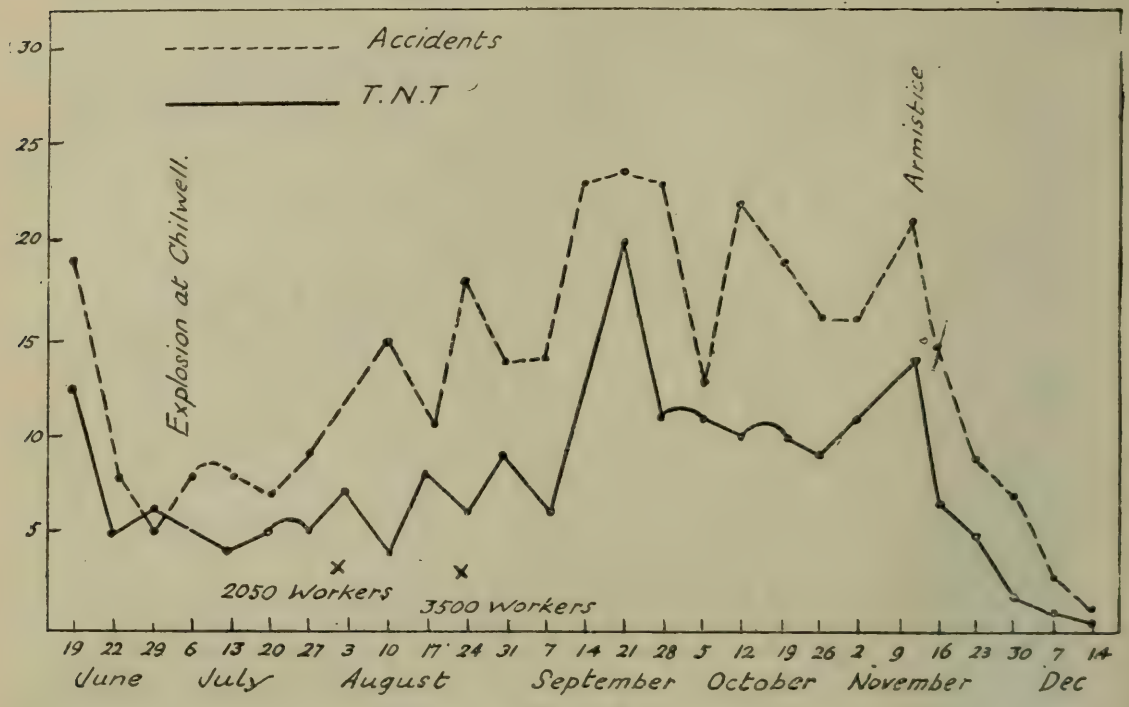


Chart C

Ventilation.

Ventilation of T.N.T. factories was introduced to effect two objects :

(1) The rapid changing of the air in which the operatives worked, and

(2) To remove dust at its point of origin by a powerful air current.

The oppression felt in any closed room where T.N.T. was manipulated in any bulk was a real phenomenon, and prolonged physical labour was depressing and enervating under such conditions, especially when an artificial high temperature was maintained to facilitate the working of ammonium nitrate.

Every T.N.T. worker is to some extent an absorber of T.N.T. and must in consequence have a microscopic amount of methaemoglobin and NO-haemoglobin in circulation, and an adequate scheme of ventilation is a remedial measure tending to preserve the balance of the haemoglobin katabolism, an undue strain of which Dr. Moore suggested as the underlying cause of the grave ill-effects of T.N.T.

Removal of dust or fume from its place of origin was effected by an exhaust draught produced by mechanical means.

An electric fan operating in a current of air carrying T.N.T. dust particles has explosive and fire risks of its own ; to eliminate this risk an *induced exhaust draught* was generally used. A powerful current of air was blown through a lateral opening at an acute angle into the main duct and this current bore along with it the air in the hinder part of the duct whose opening lay close to the working

process where the dust originated. These induced exhaust draughts were of particular value in the filling of exploder bags; minute quantities of fine dust might fall from the spigot of the bag-filling machine, but with the draught in operation none fell on the worker's hand or on the working bench, and loose empty bags that should not be exposed to possible contamination in the working area were whisked away along the duct. Processes in which T.N.T. powder was weighed out by hand were safeguarded in a similar manner.

The factory P. was a good example of the effect of ample ventilation in countering minor illness due to T.N.T. absorption; it was situated on the west coast open to the Atlantic breezes. There were four sheds with five or six melting coppers in each. The soiling of floors and benches and the splashing of workers' clothes with molten T.N.T. was common. The spigots of the melting coppers dripped continually, alternation of the employees was unreliable and there were other faults in factory hygiene. Under these bad working conditions jaundice cases were numerous over a short period—17 cases with 7 deaths in nine months July 1916 to March 1917, but the minor sickness throughout was quite a small amount, varying from 2 to 5 cases a week.

Alternation of Employment.

Alternation of employment has been another safeguard to which great importance has been rightly attached. By removing the worker from contact it lengthens the period during which the factory medical officer can pick out the incipient stages of grave poisoning, it gives time for the elimination of T.N.T. absorbed, and as shown by the table below comparing the experiences of three large and similar national factories, its existence has been a great factor in preventing a large amount of serious disease during the early period while adequate precautionary measures were being installed.

Alternated (1916).				*Not alternated* (1916).			
Factory	Workers.	Cases.	Deaths.	Factory	Workers.	Cases.	Deaths.
H.	2,011	9	2	C.	3,001	85	25
G.	1,308			L.	2,907		
A.	2,989			M.	1,593		
	6,308	9	2		7,501	85	25

It is obvious that any system of alternation at least doubles the total number of employees in contact with T.N.T., and if 'susceptibility' is a factor there is a risk of more workers developing toxic jaundice unless picked out early by the factory medical officer.

Total Cases of Toxic Jaundice from T.N.T. in Quarterly Periods.

Year.	*First.*	*Second.*	*Third.*	*Fourth.*	*Total.*
1916	6^{1}	16^{5}	73^{21}	86^{24}	181^{51}
1917	83^{12}	56^{20}	21^{1}	29^{4}	189^{44}
1918	13	6^{3}	5^{2}	10^{1}	34^{10}

Raised figures are *deaths*.)

(5) Theories of Causation of Toxic Jaundice.

In 1916 when T.N.T. work was being heavily pushed and all medical men connected with the problem were being overwhelmed by the flood of work consequent upon the vast numbers of people suddenly absorbed into this new industry, a simple aetiology was everywhere accepted as to the causation of toxic jaundice. Mr. Webster's urine test has shown that practically all workers were absorbers of T.N.T., and toxic jaundice was ascribed solely to the absorption of trinitrotoluene.

With the persistence of toxic jaundice under improved factory conditions the question of an intercurrent factor had forced itself upon the attention of all concerned in the problem, and was first publicly referred to in the meeting of the Section of the Epidemiology and State Medicine of the Royal Society of Medicine of April 12, 1918, and this view is receiving to-day more general acceptance.

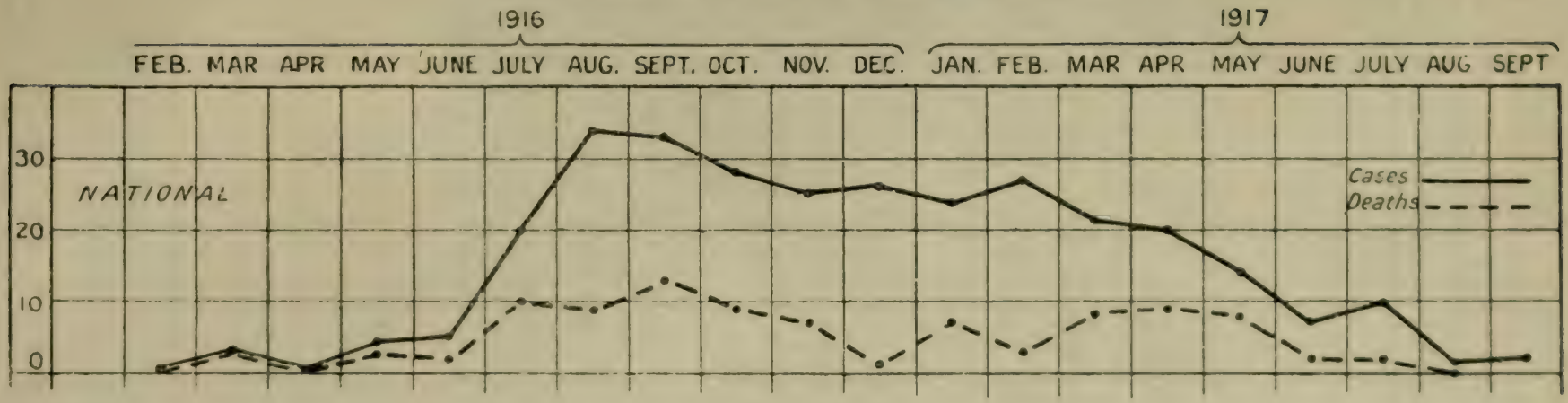
The difficulty of producing portal cirrhosis of the liver in animals by the administration of alcohol has always suggested that an infective factor was always at work in human cases, and the occurrence of cirrhosis of the liver in cows slaughtered for human consumption has always provoked the thought that alcohol was by no means an essential factor. Further, no case has yet been reported of the undoubted production of typical liver changes of jaundice in animals through the administration of T.N.T. Over 100,000 workers have at one time or other been in contact with T.N.T., and yet in all there have only been 404 cases of toxic jaundice reported to the Home Office in the whole country.

A parallel may be drawn between hundreds of thousands of intravenous injections of salvarsan that have been made and the few cases of liver atrophy that have occurred from its administration, from which Dr. Macdonald in the *B.M.J.*, January 19, 1918, favoured the view that in the causation of yellow atrophy we have at least two of the factors at work. The chart (A) printed on p. 19 is very suggestive of an acute infection. The curve below (p. 27) representing the cases of toxic jaundice occurring in the national factories shows an acute onset with a gradual decline such as is seen in curves of epidemic disease, and the Chilwell curve is very similar.

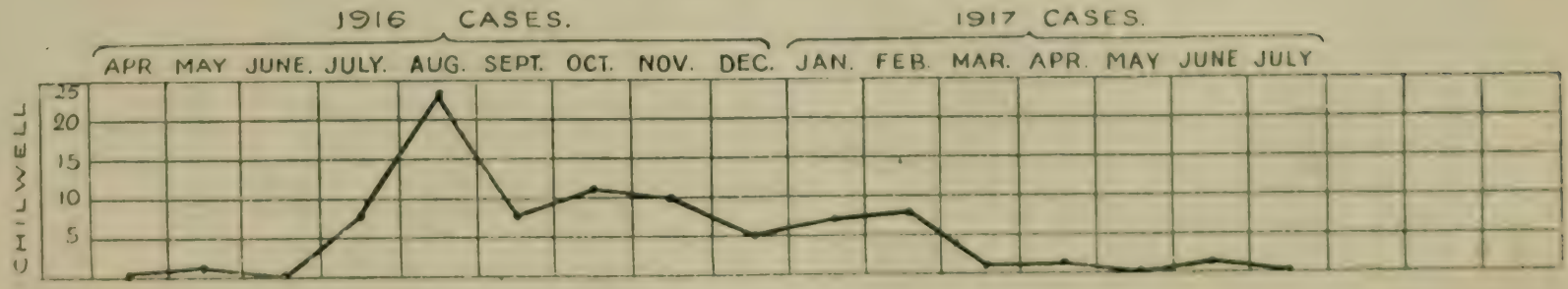
The main outbreak of jaundice cases occurred in Chilwell in August of 1916, in Leeds in September and October of 1916 ; Georgetown was first troubled with an outbreak of two cases in December 1916, and one in January 1917 ; Morecambe had an outbreak of 6-6-3-8 cases in the months of January, February, March, and April respectively, 1917, whereas Hereford had an outbreak of five cases in September, October, and November of 1918. On this one might suggest that the outbreak was comparable with a minor epidemic travelling from place to place. A further factor and one difficult to explain on the basis of a simple aetiology is the LATENT PERIOD observed in so many cases. It is now a common observation that a worker may leave a T.N.T. factory well and yet weeks or months later may be suddenly stricken with toxic jaundice.

It has to be remembered that there were always a certain number

Toxic Jaundice Cases in National Factories.



Toxic Jaundice Cases at Chilwell.



of cases of fatal acute atrophy of the liver occurring in this country, from 43 to 79 yearly, as shown by the Registrar General's returns, 1911 to 1917, and it may well be that in workers whose livers have been sensitized by the action of some metabolic product of T.N.T. bacterial infection that ordinarily would produce a gastric catarrh or a mild catarrhal jaundice would in these cases produce an extensive damage of the liver similar in kind, but much graver in degree, to that found in eclampsia and delayed chloroform poisoning.

APPENDIX

Instructions to Medical Officers of Filling Factories.

1. *Introductory.*—Men and women doctors have been attending at the various filling factories in increasing numbers since it was first realized that all persons coming into contact with T.N.T. in any shape or form could not escape absorbing it through the skin, respiratory or digestive tracts. These doctors should realize the distinction between preventive and curative medicine. They are not stationed there for the purpose of carrying out ordinary medical care of sick people. For coughs, colds, and sprains, for consumption and all other injuries, ailments and diseases there is, or should be, an available panel doctor. The medical officer, on the other hand, is appointed mainly for preventive work.

2. It is of far greater national importance that the incidence of T.N.T. sickness should be lessened, and, if possible, completely stopped, than that this illness when established, together with any other disease that may occur in the factory, should be personally treated. The medical officer should understand the working of the factory from A to Z; he should be familiar with the housing, habits, rate of wages, and the travelling facilities of the employees; he should know the relative danger of each part of the filling process, and be able to offer definite advice as to improvement in sanitation, ventilation, heating, or weatherproofing of the various factories and sheds when, in his opinion, they are defective. General suggestions are usually of less value to the management than particular and detailed advice which is the result of skilled observation and consideration.

Broadly speaking, it may be said that the principal duties of the medical officer in T.N.T. factories are five:

(1) Preliminary medical examination.
(2) Periodic routine inspection.
(3) Examination of cases.
(4) Notification.
(5) Responsibility for treatment.

All workers in the filling factories, no matter to what department of the Ministry they belong, shall be under the supervision of the medical officers.

3. *Preliminary Medical Examination.*—The first duty of the medical officer is to arrange for the preliminary medical examination

of all workers on their entrance into the T.N.T. side of the factory. The examination should be conducted in a properly lighted, warm, and sufficiently private room, with the applicant stripped to the waist and a shawl or a half blanket around the shoulders. A record must be kept on the authorized card index form, and any subsequent examination should be kept recorded on the back of the same form, or on continuation cards. The importance of this is emphasized by the fact that many conditions such as chlorosis, phthisis, mitral stenosis, aortic incompetence, chronic nephritis, and diseases of far greater rareness are now being met with in the course of this routine preliminary examination. Such cases need not necessarily be rejected as unfit for work, but if employed on T.N.T. the record of their state when first seen would subsequently facilitate a correct diagnosis and might lead to a great saving in compensation. It is advisable to weigh all employees when first seen. Epigastric discomfort and nausea, without or with vomiting, are common symptoms in T.N.T. workers, but are equally common in chlorosis, alcoholic gastritis, and dental dyspepsia. So simple an aid to diagnosis should not be neglected.

4. *Periodic Routine Inspection.*—Many employees, from patriotic or other motives, will work even though they feel far from well, and will even seek to conceal their symptoms. People have been seen at work with conjunctivae chocolate-coloured from deep jaundice. It is only by the frequent inspection of the factory by the medical officer that such cases and others suspected of illness can be suspended or transferred to other work before they either report themselves sick or collapse. Half the value of the medical officer's work is lost if he is content to wait for the employees to report themselves sick. This periodic inspection of all workers, after the initial strangeness has worn off, is welcomed by the employees as evidence of the care taken of them, and it can be rapidly and efficiently done by noticing the colour of the tongue, lips, and conjunctivae and the lack of individual activity and energy at work. Experience shows that all workers very much dislike a fuss being made about their health in the presence of other employees.

5. *Examination of Cases.*—It will be found that among the cases of sickness due to T.N.T. the main types that require absence from work are :

(1) Dermatitis.
(2) Gastritis (abdominal pain and vomiting).
(3) Severe anaemia.
(4) Jaundice of any degree.

Many doctors have found it advisable to select the more anaemic girls, or those newly employed complaining of gastric pain, and to order them to attend at the surgery two or three times a day for a week or a month to obtain a regular dose of medicine from the nurse. For want of due examination by the medical officer, cases of visceroptosis, of chronic salpingitis, of cancer of the stomach, and of hypochrondriacism have received compensation and have been seriously treated as cases of T.N.T. poisoning.

6. With careful and frequent medical inspection cases of toxic

jaundice should be rare. If such occur the medical officer should keep in close touch with their progress. Workpeople must never be allowed to leave the factory seriously ill without arrangements being made by the medical officer to keep himself informed as to the progress of the case.

7. The medical officer should attend every post-mortem and inquest whenever possible: (1) to ensure that the sectio cadaveris is adequately performed and exclude causes of death other than T.N.T.; and (2) to give evidence as to the precautions taken in the factory to protect the workers.

Owing to the fact that some of the sequelae of T.N.T. poisoning, consequent upon the fatty degeneration and atrophy of the liver cells, may not manifest themselves until some years have passed, it is particularly important that accurate and full records be kept of all sickness in T.N.T. works.

8. *Notifications.*—The Ministry of Munitions require to know the amount of sickness referable to T.N.T., the number of workers suspended or transferred to other work on account of it, and the number of cases of and deaths from toxic jaundice. A weekly return on the special form drawn up for this purpose should be sent to the Medical Adviser at the Ministry giving these details. Any case of or death from toxic jaundice should be immediately reported on its special form to the Ministry. This weekly report is not intended in any way to supersede the statutory obligation devolving on every medical practitioner attending on or called in to visit a patient whom he believes to be suffering from toxic jaundice, contracted in a factory or workshop, to notify it to the Chief Inspector of Factories at the Home Office, London (*Factory and Workshop Act*, 1901, s. 73). These latter reports form the basis of the statistics of toxic jaundice published every month in the ' Labour Gazette '.

9. *Responsibility for Treatment.*—The care of T.N.T. workers may be preventive or curative. With regard to the former a special circular will be issued embodying the principal points which need attention; with regard to the latter, whilst as a rule curative measures will be in the sphere of the panel doctor, the medical officer has a definite share in this responsibility. He should maintain good and intimate relations with the panel doctors who deal with these cases and offer such information, advice, and assistance as may seem advisable. He should also make himself responsible for seeing that the more serious cases receive adequate hospital treatment.

Any suggestions the medical officer can make as to arrangements found helpful at his own factory should be submitted to the Ministry, that they may be submitted for consideration to other factories. Any special dangers that come to his knowledge should be brought to the notice of the Ministry so that in visiting other factories similar defects in organization or care can be looked for and remedied.

10. A medical block is a necessity for the satisfactory performance of the doctor's duties and to economize the time of the workers. There should be separate waiting-rooms for men and women, sufficient privacy for undressing, and sink and water, bench and chemical reagents so that necessary tests can be carried out at once. The examination of new employees and of those with serious symptoms

should be conducted with at least as much thoroughness as obtains in ordinary hospital practice. For proper economy of the medical officer's time, it is advisable that the patient should come before him with his record card filled up as to name, age, weight, and so on; for this purpose the management may reasonably be asked for clerical assistance. At some factories branch surgeries are provided which can be used for minor dressings and treatment, but the medical officer should work at one central station where he is always available; experience has shown that this arrangement avoids confusion of records and loss of time for the worker.

The medical care of T.N.T. workers is of the utmost national importance. Men and women come to us ignorant of danger, the latter in the healthiest period of their lives; we must spare no pains and no professional skill in preventing their leaving us, injured perhaps even for life.

(Signed) W. J. O'DONOVAN, M.D.,
Chief Medical Officer.

Ministry of Munitions,
October 1916.

II. EXPERIMENTS ON THE TOXIC EFFECTS OF TRINITROTOLUENE IN ANIMALS[1]

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CONTENTS

		PAGE
	Introduction	32
(1)	Acute Poisoning	33
	Symptoms	33
	Lethal Doses	33
	Effects on Liver, Blood, and Bone-marrow	34
	Protection from Acute T.N.T. Poisoning by Oxygen	40
(2)	Chronic Poisoning	41
	Symptoms	41
	Effects on Liver, Blood, and Bone-marrow	42
(3)	Methods of Administration	43
(4)	Discussion	45
	Summary and Conclusions	48
	Acknowledgements	48
	References	48

Introduction.

In order to help in the elucidation of some of the problems offered by T.N.T. poisoning, the experiments detailed below were undertaken. They show that, in common with many nitro- and most amino-compounds, T.N.T. has a toxic effect upon haemoglobin, forming a compound no longer capable of carrying oxygen to the tissues. The fact that Webster showed that an amino (strictly a hydroxyl-amino) compound is excreted in the urine of man and rabbits dosed with T.N.T., and that this compound when set free from the glycuronic acid with which it is conjugated is more toxic than T.N.T. itself, suggests that it is the amino group formed in the organism which puts haemoglobin out of action. Such nitro bodies as picric acid (trinitrophenol), which are non-toxic, are, through some chemical or physical peculiarity, probably not reduced in the organism: dinitrophenol is very toxic, and is known to be reduced to aminonitro- or diamino-phenol.

Definite anaemia was produced by large doses of T.N.T., though not of the aplastic type. Benzene being known to cause aplastic anaemia in rabbits, the speculation suggests itself that the toluene nucleus of T.N.T. is responsible for the T.N.T. anaemia.

Liver degeneration, similar in type to, but milder in degree than, the human form, was caused by large doses of T.N.T.

[1] The experiments described in this Report were concluded in August 1917.

Factory observations and experiments having shown that the skin is a highly important channel of absorption in human beings, it is of interest that cats were shown to be capable of absorbing lethal quantities of T.N.T. by the skin.

The experiments were carried out with pure samples of the symmetrical isomer, *a*-trinitrotoluene, this being the only one used in shell-filling.

For convenience of description the paper is divided into four parts: (1) acute poisoning, (2) chronic poisoning, (3) methods of administration, (4) discussion.

(1) Acute Poisoning.

Symptoms.

Administration of large doses of T.N.T. to cats led to very pronounced symptoms, whereas rabbits and rats frequently showed very little unless a dose so large as to be quickly fatal were given.

In cats the following are seen: extreme cyanosis, salivation, dyspnoea, diarrhoea, and, if the animal lives a day or two, complete anorexia and rapid wasting.

In rabbits and rats clonic convulsions occur after very large doses, and generally, but not always, indicate approaching death. Slight cyanosis and sometimes diarrhoea occur.

Lethal Doses.

A survey of 13 experiments with *cats* shows that about 0·2 grm. per kilogram body weight is lethal in this species.

Thus, *subcutaneous injection* in oil of
0·21, 0·2, 0·2 grm. per kg. killed cats of 0·38, 2·5, 0·5 kg. wt. respectively, whereas
0·176, 0·17, 0·125 grm. per kg. did *not* kill cats of 0·42, 0·5, 0·62 kg. wt. respectively.

Administration by *mouth in oil* of
0·2, 0·185 grm. per kg. killed cats of 2·5, 0·4 kg. wt. respectively, whereas
0·17, 0·125 grm. per kg. did *not* kill cats of 0·5, 0·58 kg. wt. respectively.

Administration by *mouth of T.N.T. powder*
0·26, 0·25 grm. per kg. killed cats of 0·53, 0·51 kg. wt. respectively, whereas
0·17 grm. per kg. did *not* kill a cat of 0·58 kg. wt.

In *rabbits*, the lethal dose is between 0·5 and 0·7 grm. per kilogram body weight.

A rabbit of 1,500 grm. died three hours after a dose of 0·7 grm. per kg. Convulsions began 20 minutes after the dose. A rabbit of 1,240 grm. survived a dose of 0·5 grm. per kg. It appeared ill the day after the dose, but was well on the third day.

In *rats*, the lethal dose appeared to be somewhat higher than in rabbits.

A rat of 60 grm. died 28 hours after a dose of 0·7 grm. per kg.

A rat of 140 grm. survived a dose of 0·5 grm. per kg. No toxic symptoms occurred. Thus, 0·7 grm. per kg. is probably the lethal dose.

Effects on Liver, Blood, and Bone-marrow.

Liver. I had paraffin sections made from the livers of 14 animals (3 cats, 4 rabbits, 6 rats, and 1 monkey), dying, in periods varying from a few hours to a fortnight, of large doses of T.N.T. Except in those dying very rapidly the doses were repeated, in most cases daily.

Dr. H. E. Turnbull, Pathologist to the London Hospital, was kind enough to examine all the slides for me, and I wish to acknowledge most gratefully the great assistance he afforded me. It had been expected that large doses, single or repeated, would enable one to reproduce in animals the extremely degenerate liver found in fatal human cases of toxic jaundice. This expectation was disappointed, but in the case of four animals, one of each species, fatty degeneration of the hepatic cells at the centres of the lobules was found. The condition was stated by Dr. Turnbull to be similar to that found in early cases of T.N.T. poisoning in human beings, and in one or two rabbits fed with T.N.T. by Dr. Benjamin Moore in the early days of his work on the subject.

A brief account of the experiments on the four animals, giving a partially successful result, is here given :

Cat 32. This was a kitten weighing 320 grm. It had two injections of 0·064 grm. T.N.T. in olive oil (5 per cent. solution), one on each of two successive days. This in the ordinary way would be fatal rapidly. The animal was kept alive in an atmosphere of oxygen, maintained as nearly as possible at a concentration of 70 per cent. (see further, p. 41). The animal was kept in the oxygen chamber about 6½ hours on the first day, and 6 hours on the second. It was very weak and ill when taken out on the second day, and died during the night. Post-mortem, the liver showed to the naked eye a very striking appearance suggesting fatty degeneration. This was confirmed microscopically as stated above.

Rabbit 54. Weight 1,860 grm. Fed on carrots only. Three injections of 0·5 grm. T.N.T. in oil were given during 4 days. The weight rose a little for two days and then fell away rapidly to 1,360 grm. at death. This animal had received 19 days before the first T.N.T. injection, and 25 days before death, an injection of neo-salvarsan. The liver changes were more marked than in any of the other livers examined. There was necrosis at the centres of the lobules and fatty degeneration outside the necrotic areas. The injections of T.N.T. had been given in this case for the purpose of obtaining urine for chemical examination, but the naked eye appearance of the liver was so arresting (pronounced nutmeg type) that a piece was kept for section. Dr. Turnbull gave it as his opinion that the changes were much too acute to be ascribed to the neo-salvarsan 25 days before.

Rat 5. Weight 70 grm. White rat. Given two injections, each of 0·05 grm. T.N.T. in olive oil, with an interval of three days between the injections. Five minutes after the second injection, clonic convulsions occurred. This being nearly always a sign that death would soon occur, the animal was killed with a little chloroform. The fatty changes in the liver were slight but definite.

Monkey. Weight, 4,240 grm. This was the same animal as that referred to under chronic poisoning (see pp. 41–3), but the changes were acute ones, and must therefore be ascribed to the large subcutaneous doses given in the last few days. In the last 14 days nine doses of 0·5 grm. T.N.T. were given hypodermically in oil and a final dose of 0·75 grm.

In the other ten livers examined the only noticeable changes were slight catarrh of the large bile ducts in a cat (12 days' dosing by the skin), and proliferation of the connective tissues round the bile ducts in a rabbit (16 days' injections). In the latter no evidence was found macroscopically or microscopically of coccidiosis, a condition which frequently causes such changes. The remaining eight livers showed no changes of importance.

Besides these 14 livers there were several cases, where for one reason or another no microscopic examination was made, in which a pathological condition would have been almost certainly found. In particular two rabbits showed pronounced nutmeg livers. One of these two had had novarseno-billon 11 days before his first dose of T.N.T. It had five doses of 0·7 grm. T.N.T. in oil subcutaneously, spread over seven days; it died on the ninth day. The other had had no previous treatment. It had five doses on five consecutive days, the first of 0·5 grm., the second of 0·6 grm., the remaining three each of 0·7 grm., subcutaneously in oil. The animal died on the seventh day.

Blood. The effect of T.N.T. on *haemoglobin* varies considerably with the species of animal. Cats show distinct cyanosis with small doses and extreme cyanosis with large doses. Lethal doses kill entirely by oxygen-want. Rabbits and rats require much larger doses than cats before any change in the colour of the blood-pigment is obvious. Large doses cause the appearance of a chocolate-coloured pigment in the blood, and lethal doses probably owe their effect again to asphyxia, the convulsions referred to above being almost certainly asphyxial in origin. The chemistry of the pigment formed is somewhat uncertain, but it is perhaps identical with that formed by the action of nitrites on haemoglobin (Haldane (2)), which may for convenience be called nitrite-haemoglobin. According to Haldane this is identical colorimetrically and spectroscopically with a mixture of one part nitric oxide haemoglobin and three or four parts of methaemoglobin. Methaemoglobin was looked for spectroscopically many times in human beings and in animals. The characteristic band in the red was found only in one case, that of a cat given 0·5 grm. T.N.T. in oil by stomach tube. The animal died in three hours, and the sample of blood giving the methaemoglobin spectrum was taken from the saphena vein shortly before death. The band in the red was faint even in this case, though the blood must have been very heavily loaded with 'nitrite-haemoglobin'.[1]

Haldane Test. The test-tube method of laking blood with water and saturating with coal-gas or carbon monoxide was used in several cases. It gave positive results (slight turbidity and a purplish tint

[1] It is difficult to reconcile these spectroscopic observations with any theory that the main change is to methaemoglobin, and additional experimentation upon the altered haemoglobin is evidently desirable.

as compared with the cherry red of the control) in cats and rabbits, in several munition-workers using T.N.T., and in two laboratory workers who were taking daily doses of 0·05 grm. T.N.T. experimentally for a week. The *serum* was examined in Wright's tubes in two cases. One was that of a cat having a series of daily doses of T.N.T. by the skin, the concentration of T.N.T. in the blood being small as judged by the degree of cyanosis ; the other was a rabbit having a series of large doses hypodermically. In neither case was haemolysis or bile present. At the post-mortem on the monkey, which was made immediately after the animal had been killed, the blood was noticed to be very distinctly haemolysed. The last dose, two days before death, had been a very large one. In no case was haemoglobinuria observed. This last fact is somewhat remarkable in contrast with some unpublished observations of Dr. Moore's, viz. that rabbits fed with large doses (1 grm. or more) of T.N.T. in powder-form, almost invariably got haemoglobinuria a day or two before death. The difference is presumably due to the different methods of administration, the great majority of my rabbits receiving subcutaneous injections of oily solutions. Evidently the route of administration is a more decisive factor than previous solution of the substance in setting up a high concentration in the blood.

With regard to the *blood-cells*, only a few desultory observations were made until a fortnight before the author was called up for military duties. These early observations were limited to counts of the red cells in three cats and one rabbit ; all showed a definite fall in the number of red cells while large doses were being given, and in the case of the rabbit a tendency to rise again on stopping the doses.

A fortnight before the work had to be abandoned an observation was made on a rabbit's blood which led to a hurried series of experiments, the results of which were of considerable interest. The observation referred to was made while examining a blood film for a differential leucocyte count, the latter being undertaken after reading a paper by Selling, showing that benzene in daily doses of 1 c.c. produces in rabbits within a week an enormous fall in leucocytes, and affects the polymorphonuclear cells much more than the mononuclear. That is to say, that the condition of the white cells in benzene poisoning is the same as that found in aplastic anaemia. Quite a number of cases of aplastic anaemia had by this time been reported in munition-workers handling T.N.T., so it was determined to try whether large daily doses of T.N.T. could produce changes in the direction of those found in aplastic anaemia.

Before recording what was found it may be as well to recall the salient features in the blood-picture in a human case of aplastic anaemia ; these are (according to Musser (4)) : red cell count very low, megalocytes and erythroblasts absent, no poikilocytes, no anisocytosis, haemoglobin low, colour index variable but mostly low, great leucopenia affecting especially the polymorphs.

The findings in the T.N.T. rabbit (No. 34) were as follows : after six doses (first two of 0·4 grm. each, last four of 0·5 grm. each) in ten days the red cells showed very pronounced polychromatophilia, anisocytosis, poikilocytosis, and the presence of nucleated forms ; the red-cell count (see Fig. 1) was low, 3,360,000 (normal being 4,500,000

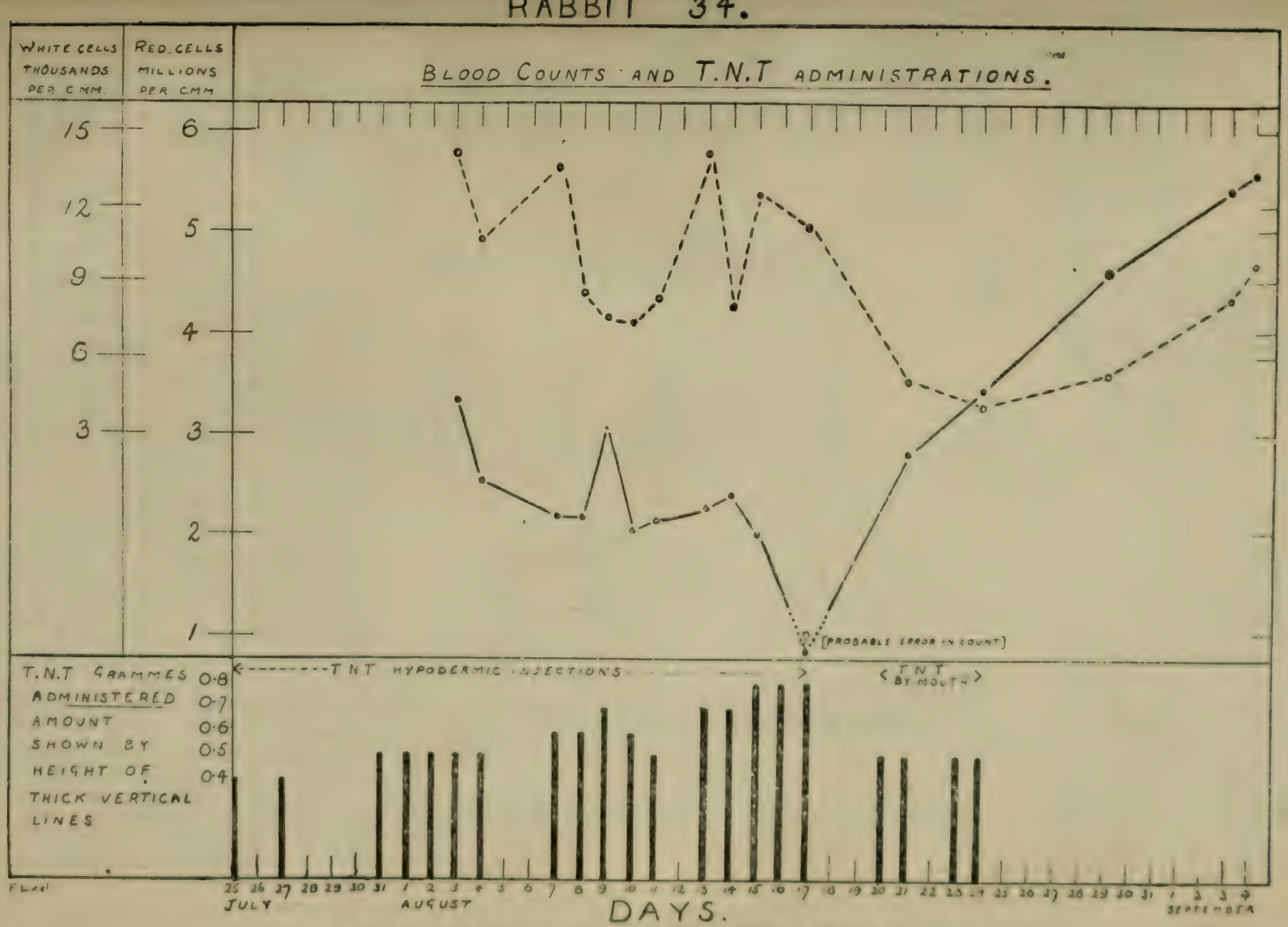


FIG. 1. (*Red cells continuous line; white cells broken line.*)

to 7,500,000) per cubic mm.; the white-cell count was rather low, but subsequent observations showed it to be fluctuating, the differential count also showed fluctuations. Injections and blood observations were continued for three weeks (Dr. Moore very kindly continued the injections for a time after my departure, and sent me blood films from time to time), and the blood-picture was found to remain as described above, except that the nucleated reds diminished considerably. The animal was then left untreated. Eleven days later the red-cell count was 5,584,000, and the film was practically normal, no nucleated red cells being seen, and only very slight polychromatophilia and anisocytosis. The dosage in this case, as

Blood counts of Rabbit 34.

Date.	*Dose of T.N.T.*	*Red cell count.*	*White cell count.*	*Nucleated red cells.*
July 25	0·4 grm. subcutaneously	..	..	Number seen
27	0·4 ,, ,,	..	..	while counting
31	0·6 ,, ,,	..	..	200 leucocytes.
Aug. 1	0·6 ,, ,,	..	..	
2	0·6 ,, ,,	..	..	
3	0·6 ,, ,,	3,400,000	14,000	
4	0·6 ,, ,,	2,600,000	11,000	
7	0·8 ,, ,,	2,100,000	13,000	
8	0·8 ,, ,,	2,200,000	8,000	3
9	0·8 ,, ,,	3,000,000	7,800	7
10	0·6 ,, ,,	2,050,000	7,800	22
11	0·4 ,, ,,	2,200,000	8,100	
13	0·6 ,, ,,	2,300,000	14,200	8
14	0·6 ,, ,,	2,400,000	8,200	14
15	0·8 ,, ,,	2,100,000	12,500	
16	0·8 ,, ,,	..	..	4
17	0·8 ,, ,,	..	11,000	1
20	0·5 ,, by mouth	..	..	
21	0·5 ,, ,,	2,700,000	5,000	
23	0·5 ,, ,,	..	..	
24	0·5 ,, ,,	3,500,000	4,000	1
	No more T.N.T.			
29		4,300,000	4,800	
31		..	5,100	
Sept. 3		5,200,000	8,000	
4		5,400,000	9,500	0

usual, was by hypodermic injection of olive-oil solutions of T.N.T., the doses being given daily except on Sundays. The dose was increased gradually until 0·8 grm. was reached; this was given on three successive days. Since a strength greater than 5 per cent. cannot readily be used, and even this requires heating to dissolve the T.N.T., 5 per cent. solutions were always used. This necessitated the employment of fairly large quantities of oil, hence it was thought advisable to do a control experiment with olive oil alone, in order to eliminate the possibility that some or all of the effects were due to the unsaturated fats (see St. Faust and Tallquist (6)) of the olive oil. The control received doses of hot olive oil almost daily for three weeks (17 doses in 21 days), the quantity of oil being the same as that used in the T.N.T. experiment. Blood films were examined on two days before injection, on several occasions during the experiment, and on the last day. The red cells were on all these occasions normal in shape and staining; no nucleated red cells were ever seen. The size of the cells were normal and uniform at first, and

showed only a very slight anisocytosis towards the end. A blood count on the thirteenth day gave 5,332,000 cells per cubic mm., in contrast with 2,240,000 in the T.N.T. rabbit on the twelfth day.

In a third rabbit (No. 39), the blood was examined *ab initio*. On the two days before injection the red-cell counts (see Fig. 2)

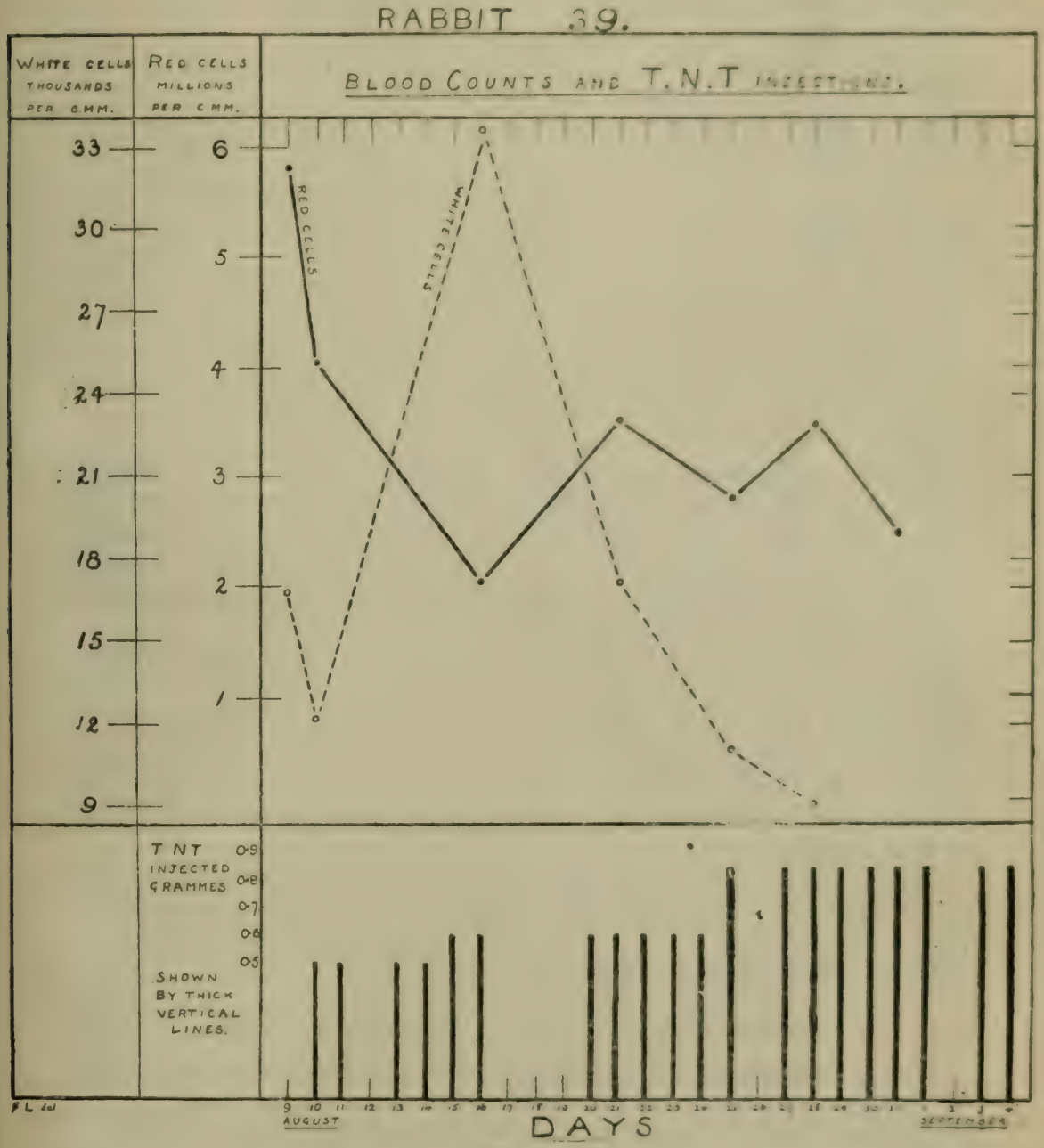


Fig. 2.

were 5,870,000 and 4,120,000 respectively, and the film was normal. After four doses of 0·5 grm. T.N.T. and one of 0·6 grm., slight polychromatophilia, slight anisocytosis, and twelve nucleated reds were seen while counting 200 white cells. The red-cell count was 2,070,000. Dosing was continued for three weeks. The red-cell count remained low and the film similar, except that the nucleated reds diminished in number considerably.

Blood counts of Rabbit 39.

Date.	*Red Cells.*	*White Cells.*	*T.N.T. Injected.*	*Weight.*
	Millions.	*Thousands.*	*Grammes.*	*Kilograms.*
Aug. 9	5·87	16·85	..	2·16
10	4·12	12·20	0·5	
11	..	..	0·5	2·30
13	..	..	0·5	2·26
14	..	..	0·5	2·28
15	..	..	0·6	2·24
16	2·07	33·60	0·6	
17	..	..	..	2·20
20	..	..	0·6	
21	3·50	17·16	0·6	
22	..	..	0·6	2·30
23	..	..	0·6	
24	..	..	0·6	
25	2·88	11·25	0·85	
27	..	..	0·85	
28	3·43	9·00	0·85	
29	..	..	0·85	
30	..	..	0·85	2·35
31	2·47	..	0·85	
Sept. 1	..	..	0·85	
2	..	..	..	
3	..	..	0·85	
4	..	..	0·85	

These results show that T.N.T. causes in rabbits both red-cell damage and destruction (staining changes and low blood counts), and stimulation of the blood-forming organs (presence of nucleated reds in peripheral circulation). Evidence was also obtained tending to show that regular daily dosing with still larger quantities would produce even more profound effects.

It will be noted at once that the blood condition obtained was far from identical with that found in aplastic anaemia, but it is quite conceivable that blood destruction and bone-marrow stimulation, if continued long enough, would lead ultimately to bone-marrow exhaustion, which seems to be the essence of aplastic anaemia. The diminution of nucleated red cells as the poisoning progressed points in this direction.

Bone-marrow. This was examined in only one acute case, that of the monkey referred to previously. The marrow of the femur was red throughout. Microscopically it showed the presence of numerous cells of all the usual varieties.

Protection from Acute T.N.T. Poisoning by Oxygen.

The symptoms following large doses of T.N.T. in cats pointed so plainly to asphyxia as the chief, if not the only, element causing their rapid death, that it was thought worth while to try whether life could be prolonged by placing the animal in an atmosphere of oxygen, on account not only of its intrinsic interest, but because it was hoped that life might thereby be extended long enough for liver changes to be produced. Kittens were used for this purpose, weighing half a kilogram or less. The method adopted was to inject subcutaneously a large dose (one that would have been fatal in a few hours in the ordinary way) and immediately place the animal in a chamber through which oxygen was passed rapidly from a cylinder until its partial pressure was very high ; then this

partial pressure was maintained at the required level by slow bubbling of oxygen. The apparatus used was very similar to that used by Adams (1) in his work on tuberculosis and oxygen.

Of seven kittens used in this manner, three survived the first dose twenty-four hours or more, and were given a second dose, one, two, and three days respectively after the first dose; all these three died within twelve hours of the second dose. Three others died six, twelve, and twenty-four hours respectively after their first dose, in all cases later than would probably have been the case if no oxygen had been used. (One control left under ordinary conditions died in three hours.) The seventh kitten died in 3½ hours, the only one to show no result; this was a weakly kitten to start with, and the weather was very hot at the time.

Thus in six out of seven cases a definite prolongation of life was effected by the use of oxygen, and in three a single lethal dose was recovered from, though a second proved fatal, probably because it was given too soon after the first, though in two or three cases, when the lungs were examined microscopically, commencing oxygen-pneumonia was present. In six of the seven cases an attempt had been made, following Adams, to keep clear of oxygen-pneumonia by maintaining the oxygen percentage at 70 or a little higher.[1] According to Adams, animals can survive for lengthy periods in an atmosphere containing 70 per cent. oxygen or less. The above results do not seem to bear this out, but of course the T.N.T. complicates the question.

With regard to changes in the liver, only two of these seven were examined histologically. One was 'Cat 32' (p. 34), and showed early hepatic degeneration; the other through naked eye a 'nutmeg liver', showed nothing abnormal microscopically.

On the whole the impression was obtained that the experiments would have been worth extending had time permitted.

(2) Chronic Poisoning.

Symptoms.

Eight prolonged experiments were made with cats and rabbits (three cats and five rabbits), and one with a monkey, extending over three or four months in some cases; small doses being given daily, on alternate days or less often in some cases at certain times when the effects of the poision became too pronounced.

In cats the symptoms were similar to those detailed under acute poisoning—slight cyanosis was nearly always present, slight diarrhoea often occurred. Loss of appetite and weight, if not immediately countered by withholding the dose until recovery, nearly always meant rapid wasting and death.

In rabbits there were commonly no symptoms at all, and the impression was irresistible that rabbits could deal successfully with

[1] The oxygen percentage was determined on two or three occasions each day by means of Hempel's bulbs. It should have been stated above that the injections were done in the morning and, except in the first case, the animal was always taken out of oxygen in the evening after 6 or 7 hours' exposure.

small doses of T.N.T. for an indefinite time, just as the majority of human beings in the shell-filling factories are apparently able to do. The monkey corresponded in this respect to rabbits and man.

Effects on Liver, Blood, and Bone-marrow.

Liver. The livers of three cats and two rabbits were examined microscopically. The numbers are small, but the general conclusion seems to be that there is a tendency to a mild cholangitis where the doses are not too small.

Cat 15. For four months small doses of T.N.T. were applied to the skin, at first daily, later on alternate days or less frequently. For the first $3\frac{1}{2}$ months the application was made in solution in acetone, the quantity of T.N.T. used varying from 0·1 to 0·5 grm. During the whole of this period the weight, with insignificant fluctuations, kept steady, and the general health was unimpaired. Then application of 0·1 grm. T.N.T. in lanolin was substituted. After four doses of this on consecutive days the animal was so ill that administration was stopped, but despite this, rapid deterioration continued, and death took place a fortnight after the lanolin was commenced, the weight in that short period falling from 2,320 grm. to 1,380 grm. The liver microscopically showed nothing abnormal.

Cat 18. This was a kitten weighing 590 grm. when the experiment was started. Hypodermic injections in oil were given at intervals of one, two, or three days for fifteen weeks. For the first fourteen weeks the dose of T.N.T. varied from 0·01 grm. to 0·025 grm. Then two doses of 0·04 grm. with three days between them caused rapid wasting, and death took place two days after the last dose. The liver microscopically showed nothing abnormal except some scattered deposits of iron-containing pigment.

Cat 12. This was a kitten weighing 580 grm. at the outset. On January 31 a dose of 0·075 grm. T.N.T. dissolved in oil was given by stomach tube. Nothing more was done till February 27 (four weeks) when hypodermic injections of 0·015 grm. T.N.T. in oil were commenced. Six doses were given in eleven days. Anorexia and loss of weight ensued, and the animal was killed six days after the last dose, as death was obviously impending. The liver microscopically showed cellular infiltration of the portal systems.

Rabbit 8 a. Nine hypodermic injections of T.N.T. in olive oil were given in eighteen days, the total dosage being 4·5 grm. Five weeks after the last dose the animal suddenly got convulsions and died. The liver was small; microscopically it showed excess of fibroblasts in the portal systems and some fibrosis.

Rabbit 5. Thirteen applications were made on the skin in twenty-five days, the first four being 0·5 grm. T.N.T. in lanolin, the remaining nine the same quantity in acetone. Then a single dose of 1·0 grm. was given, and a week later two small doses with a two days' interval between were given with a view to testing whether the urine reactions corresponded in intensity to those found after similar doses in human beings. They were found to be roughly comparable. Four months after the last dose the animal was fat and well. It was killed and the liver examined. Naked eye a slight coccidiosis was observed. Microscopically cholangitis and some fibrosis of the

portal systems was observed. This may have been due in whole or in part to the coccidiosis.

Another *rabbit* (No. 38) may be included here, though the length of the experiment would place it as intermediate between the acute and the chronic types. In sixteen days nine doses were given hypodermically, varying from 0·2 to 0·5 grm. Diarrhœa and wasting set in and the animal was killed the day after the last dose. The liver showed no coccidiosis, naked eye or microscopically. Proliferation of connective tissue round the bile-ducts was seen.

A *monkey* was given frequent applications on the skin of T.N.T. in acetone over a period of four months. Absorption of a certain amount took place since the urine test for T.N.T. gave a positive result the whole time, but the animal remained quite well. Finally, large doses were given subcutaneously. The liver showed no changes that could be ascribed to chronic poisoning (see p. 35).

Blood. In a rabbit slight cyanosis and a faintly positive ' Haldane test ' were present in blood taken four days after a large dose of T.N.T. (0·66 grm.). In man and the cat both cyanosis and a positive Haldane test are present after quite small doses.

The serum of *cat* 15 (see p. 42) was examined in a Wright's tube while under treatment ; no haemolysis and no bile were seen. No fall in the red-cell count was found in a cat having small doses of T.N.T. subcutaneously. Slight anisocytosis, one nucleated red cell, and two polychromatophilic cells were seen in a film taken from a thoracic vein at the post-mortem on Cat 18 (see p. 42).

Bone-marrow. At the post-mortem on Cat 18 the femur, humerus, one rib, and a lumbar vertebra were examined. They all showed red marrow throughout.

(3) Methods of Administration.

In all but a few of the experiments, administration was either by subcutaneous injection or by application to the skin. The solvent for the injections was in all cases olive oil, the strength being generally 5 per cent. To effect solution in this percentage the oil had to be heated and the injection given while the temperature was still somewhat above body temperature. Where small doses only were to be injected a strength of 2·5 per cent. was used ; heat is required here to effect solution, but the T.N.T. does not crystallize out on cooling. Absorption took place rapidly from the oily solution ; this was shown not only by symptoms, but by the fact that at the post-mortem on one rabbit some unabsorbed oil failed to give the colour test for T.N.T. with alcoholic potash.

Administration by the skin was used for two reasons (*a*) because it seemed to be probably the best method of ensuring a continuous entrance into the circulation of small doses of the poison over long periods of time ; (*b*) because experiments in shell-filling factories by Dr. Benjamin Moore (3) and myself had shown the paramount importance of skin-absorption under factory conditions, and it was desirable to establish not only that T.N.T. could be absorbed by the skin (already proved in human experiments) but that fatal doses of T.N.T. could be taken in by the skin. This was shown to

be the case by three experiments with cats. The first of these was a cat (No. 8), weighing 2,400 grm. It received nine doses of 0·5 grm. T.N.T. per dose in lanolin on the skin in thirteen days. Diarrhoea and cyanosis appeared on the third day. Rapid wasting set in and continued till death, when the weight was 1,420 grm.

The second was a cat (No. 14) weighing 2,140 grm. It received four doses only, each of 0·5 grm. T.N.T. in lanolin, on the first, fourth, fifth, and seventh days respectively. It died on the eleventh day. Cyanosis appeared on the fifth day. Wasting set in after the first dose and continued till at death the weight was 1,440 grm. The red-cell count was 5,240,000 per cubic mm. a few hours before death —the normal being 8,000,000 to 10,000,000. The stomach contents were tested post-mortem for T.N.T. and found free, showing none had been swallowed, though it was still present on the skin.

The third cat (No. 15) has already been referred to under Chronic Poisoning.

All precautions were taken in these skin experiments to prevent the animal licking the area of skin used. A coat of stout American cloth, completely covering the trunk and laced up along the back, was fitted, the limbs passing through four holes on the ventral aspect of the garment. The patch of skin used was about 3 inches by 3 inches in size and placed at the middle of one side of the trunk. At first the hair was removed by shaving; later it was found best to be content with clipping it with scissors. It was a matter of some surprise how little resentment the animals, particularly cats, showed to wearing the coat. Lanolin or acetone was used as the solvent in all the skin experiments. The former was far the more effective in promoting absorption of T.N.T., but from its messiness was not well adapted for prolonged experiments. The superior effectiveness of lanolin was almost certainly due to its remaining for an indefinite period in contact with the skin and not to its chemical nature. Acetone evaporated rapidly and left the T.N.T. in powder form, some of it sticking to the skin, but some of it not doing so. For the short period, five minutes or less, during which the acetone remained, absorption was most probably more rapid than in the case of lanolin. A series of experiments was begun, but unfortunately never completed, to test various solvents in relation to their capacity to favour absorption of a dissolved substance by the skin. The leading idea was, after a few preliminary experiments with the ordinary organic solvents, to test the various fats, fatty acids, and alcohols that occur in human skin,[1] using atropine as the dissolved substance and mydriasis as the sign of its having been absorbed. The *modus operandi* was to place one hind foot of a kitten in a wide-mouthed glass vessel containing the solution and hold it in position for a given period facing a well-lit window. The experiments were interrupted when four organic solvents and one other substance had been tested to the extent of giving positive results, and four other substances had so far given only negative results. The results may be summarized thus: the following favour absorption, the first more than the second and so on in descending order—chloroform, ether, xylene, acetone, olive oil; the following all favour absorption a good deal

[1] Or that might come into contact with the skin in munition factories.

less than does olive oil, but cannot be placed in order because only negative readings were obtained with the strengths and times used —alcohol, distilled water, oleic acid, palm oil.

The only points of interest here are that an unsaturated oil (olive oil) favoured absorption more than its corresponding fatty acid, more than a closely related saturated oil, and more than water.

The experiments were suggested in part by an account of some work done by A. D. Waller (9) in 1869 from a different point of view.

The following table summarizes my experiments :

Solvent.	*Strength of Solution.* 1 in	*Time of exposure.* *minutes.*	*Result.*
Chloroform . . .	1,000	10	Positive.
,, . . .	5,000	10	Negative.
Ether	2,000	10	Negative.
,, ...	1,000	10	Positive.
Xylene	250	20	Positive.
Acetone. . . .	1,000	10	Negative.
,, ...	500	10	Negative.
,, ...	250	25	Positive.
Olive Oil . . .	500	10	Negative.
,, . . .	250	10	Negative.
,, . . .	125	25	Positive.
Oleic Acid . . .	100	25	Negative.
,, . . .	50	25	Negative.
Alcohol	100	25	Negative.
Water	50	25	Negative.
Palm Oil . . .	50	60	Negative.

It will be observed that the saturated palm oil (mainly consisting of a glyceride of palmitric acid, $C_{16}H_{32}O_2$) in 2 per cent. solution gave a negative result after 60 minutes exposure, whereas the unsaturated olive oil (mainly glyceride of oleic acid $C_{18}H_{34}O_2$) in 0·8 per cent. solution gave a positive result after 25 minutes exposure. This difference is presumably due to difference in solvent power of the two liquids, and therefore difference of surface tension, which latter is of great importance in relation to absorption by living cells.

It was intended to investigate in this manner stearin and stearic acid, cholesterin and lanolin (which consists of esters of cholesterin) ; formic, acetic, proprionic, normal butyric, and caprylic acids, all of which acids may occur in human sweat. If results of any interest had accrued, the solubility of T.N.T. in these solvents and the surface tension of the solutions would have been determined. It was hoped that in this manner some clue would have been found to the problem of the causes of the great differences between the absorption powers of the skins of various human beings.

(4) Discussion.

The fatal forms of T.N.T. poisoning in human beings are (1) ' toxic jaundice ', a severe hepatic degeneration somewhat like acute yellow atrophy of the liver ; (2) aplastic anaemia.

The majority of the toxic jaundice cases occur after about two months' exposure to T.N.T. Several chronic experiments with animals lasting three or four months have failed to reproduce this

condition, the dosage being from $2\frac{1}{2}$ to 4 times [1]the maximum dose (per kilogram body weight) that a munition worker can probably take in daily, according to the factory observations of Dr. Moore and myself.

On the other hand, definite liver degeneration (but no jaundice) similar in type to that found in early human cases of toxic jaundice has been caused in animals by large doses of T.N.T., doses much larger than any munition workers are likely to be getting under factory conditions. The very extensive destruction of liver tissue found in human cases has not been attained in these experiments.

It is instructive to compare the cases of T.N.T. with those of other liver poisons, e. g. alcohol, chloroform, tetrachlorethane. The two latter, when they produce recognized liver lesions at all in human beings, produce them rapidly, whereas alcohol produces its effect, if at all, only very slowly. The chloroform and tetrachlorethane conditions are easily reproduced in animals by large doses (see Stiles and Macdonald for $CHCl_3$ and Willcox for $C_2H_2Cl_4$). It is, on the other hand, correct, I believe, to say that alcoholic cirrhosis of the liver has never been reproduced in animals by prolonged administration of small doses, but that it can be produced by combining the inoculation of certain bacteria with the administration of alcohol (Adami (12), Ramond (5)). Whether the latter observation be confirmed or not, the admitted difficulty in producing ' alcoholic cirrhosis ' by administration of alcohol obviously suggests that a second factor is at work in human cases. May not the same be true of the toxic jaundice ascribed to T.N.T. ? As possible adjuvant factors the following suggest themselves : bacterial activity, some inherent metabolic defect, a deficiency in diet,[2] or the presence in excess of some substance in the diet either toxic itself or productive of a toxic substance in the alimentary tract. A case described by Dr. Feldman at the Royal Society of Medicine in February 1917 is strongly suggestive of the ' second factor ' hypothesis. The case was that of a woman worker at a T.N.T. factory who stayed away from work eight weeks, and at the end of that time began to suffer from acute gastric symptoms. Two or three days later jaundice manifested itself. If I remember rightly, the patient recovered after a somewhat severe attack of jaundice, too severe to be ascribed to ordinary catarrhal jaundice. The suggestion is that the liver had already been damaged to a considerable extent but was able to cope with all ordinary demands. A severe gastroduodenitis upset the balance and led to manifest signs of the existing liver disease.

With regard to aplastic anaemia, the same hypothesis may be put forward. The observations made on the blood of rabbits on large doses of T.N.T. show distinct blood destruction and marrow stimulation. This, if continued, might lead to marrow destruction,

[1] Thus, e.g., cat 18 received doses on alternate days of 20 to 30 mg. per kg. corresponding roughly to 1 to 1·5 grm. on alternate days for a person weighing 50 kg., or, say, 0·5 to 0·75 grm. per day. Analyses of workshop air and workers' urines indicate quantities of the order of 0·1 to 0·2 grm. daily as the *maximum* amount taken in by all routes under the worst conditions and in cases with the most absorptive skins. This is an outside estimate, a more likely figure being 0·075 grm. for some of the worst cases.

[2] E.g. carbohydrate-starvation predisposes to ' delayed chloroform poisoning '. See also Rabbit 54, p. 34.

the essential feature of aplastic anaemia. But here again the results were obtained with doses far larger than munition workers could possibly be absorbing, and a second factor hypothesis usefully comes to the rescue.

The relative rarity of toxic jaundice amongst munition workers (somewhere about 1 in 200 T.N.T. workers have, I believe, suffered from the disease) and the still greater rarity of aplastic anaemia is in favour of this explanation of the very partial reproduction of the human disease in experimental animals.

Other possible hypotheses are :

1. That much larger quantities of T.N.T. are being absorbed by some munition workers than are indicated by the colour reactions in their urines. On this hypothesis a large fraction of the T.N.T. absorbed is altered in the body to a much more toxic substance whose end-product in the urine has not yet been detected.

2. That the human cases are due, not to α-T.N.T., but to impurities in commercial T.N.T., such as β- and γ-T.N.T., dinitrotoluenes, nitro-benzenes, nitrous or nitric anhydride, or even some as yet unrecognized gaseous substance formed from molten T.N.T.

With regard to (1), an apparently strong point in its favour is that cats dosed with T.N.T. do not give the urinary colour reaction which man, the monkey, and the rabbit give, while at the same time cats are far more susceptible to poisoning by T.N.T. than the other species.

In this connexion it is of considerable interest that I found that the dinitrotolylhydroxylamine, which Webster showed was the form in which T.N.T. is excreted in human and rabbits' urine, has a lethal dose for cats about one-third of the lethal dose of T.N.T. ; for rabbits its lethal dose is about three-quarters that of T.N.T. Webster further showed that dinitrotolylhydroxylamine in human and rabbits' urine is linked up with glycuronic acid, and apparently thereby rendered innocuous. These facts favour the idea that cats form first dinitrotolylhydroxylamine, which, failing to be neutralized, exerts a very powerful toxic action on haemoglobin, and is further changed in this reaction with haemoglobin into an unknown substance, whereas man and the rabbit combine the hydroxylamine, as it is formed, with glycuronic acid, thus neutralizing or at any rate lessening [1] its affinity for haemoglobin.

The question whether T.N.T. is excreted quantitatively as hydroxylamine should permit of experimental test. As a matter of fact, urine for this purpose was more than once collected, and each time through some mistake spoilt or lost. During the course of a large number of human and rabbit experiments undertaken from another point of view, no indication appeared of any failure of correspondence between the dose of T.N.T. taken and the colour reaction in the urine.

As to (2) it may be said, firstly, that no evidence has yet been brought forward incriminating any other substance present as impurity in commercial T.N.T. or formed from it in the process of shell-filling ; secondly, the various mixed impurities have been tested by Dr. Moore (3, p. 39), and found no more toxic than T.N.T., and thirdly, that a large proportion of the cases of toxic jaundice in munition workers occurred among those handling the very pure form of T.N.T. used for filling exploder bags.

Summary and Conclusions.

1. *a*-T.N.T. is very toxic to cats, moderately toxic to rabbits, rats, and monkeys.

2. The cat dies after fairly small doses from loss of oxygen-carrying capacity of the blood due to the formation of a derivative of haemoglobin lacking the power of attaching oxygen. The life of a cat injected with a lethal dose can be prolonged by placing the animal in an atmosphere of oxygen; such a dose may even be recovered from altogether.

3. Hepatic degeneration can be produced in all the four species by large doses of T.N.T. In no case has it been produced by chronic poisoning with small doses.

4. Certain blood changes can be produced in rabbits by administration of large doses which probably represent an early stage of aplastic anaemia.[1]

5. Fatal poisoning can be produced in cats by administration of T.N.T. by the skin.

6. Comparison is made with poisoning by alcohol, chloroform, and tetrachlorethane, and the general conclusion drawn that an unknown second factor probably co-operates with T.N.T. in the production of the fatal forms of 'T.N.T. poisoning' in human beings.

Acknowledgements.

A debt of thanks is due to the Medical Research Committee, on whose behalf the work was done.

It is a peculiar pleasure to place on record my warmest thanks to Dr. Benjamin Moore, F.R.S., whose assistant I was privileged to be, and who throughout gave every possible encouragement and advice.

Grateful acknowledgements are also due to Dr. H. H. Dale, F.R.S., in whose laboratory much of the work was done, and whose hospitality, advice, and assistance were most ungrudgingly given.

Special thanks are also due to Dr. H. Turnbull, whose valuable help is referred to in another part of the paper, and to Mr. T. A. Webster for very numerous occasions on which advice was given and work lightened by his co-operation.

[1] The frequent occurrence of cyanosis in munition workers and its presence in rabbits on large doses of T.N.T. show that *some* action on haemoglobin takes place either before or after the formation of the glycuronate, most probably before.

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III. ON THE METABOLISM AND EXCRETION OF 2:4:6-TRINITROTOLUENE

By T. A. Webster

Previous to the war only comparatively small amounts of symmetrical trinitrotoluene—for brevity called hereafter T.N.T.—were manufactured on a commercial scale, and as it gave rise to no toxic symptoms among the workers its pharmacology has been very little studied.

Prosser White (1) found that while 90 minims of a 1 per cent. solution of dinitrobenzene, administered hypodermically, was rapidly fatal to a cat, a similar dose of T.N.T. proved quite innocuous. He concluded that T.N.T. was non-toxic.

Jaffé (2) studied the behaviour of ortho-, meta-, and para- mononitrotoluenes in the dog, and found that while the ortho- compound was excreted in the urine conjugated with glycuronic acid, the meta- and para- compounds were found in the urine united with glycocoll and with hippuric acid. The first question studied in this research was how to detect small amounts of T.N.T. or its derivatives that might be found in the urine of workers exposed to the dangers of T.N.T. poisoning. As is well known, many of the nitro derivatives of benzene and its homologues give rise to characteristic colours when treated with a caustic alkali in alcoholic solution, and it was found that while neither the urine itself, nor an extract from it with ether or other non-miscible organic solvent, gave any colour reaction with alcoholic potash, yet if the urine was just strongly acidified with a mineral acid and then extracted with ether, this ether extract gave a pronounced colour with alcoholic potash.

The isolation and identification of this chromogenic body has been previously and briefly described in Professor Moore's (3) monograph on T.N.T. poisoning. A somewhat fuller and amended description is given here.

A priori the most obvious way for the organism to deal with T.N.T. would be either to reduce one or more of the nitro groups to amino groups and excrete the amino body so formed as a salt, or to oxidize the methyl group and eliminate the acid.

The first attempts at isolating the chromogenic body were made with human urine, as it was not quite certain that animals dosed with T.N.T. excreted it in the same form. The urine of a large number of workers from the shell-filling department of Woolwich Arsenal, some 12 gallons in all, was collected. Owing to the large bulk of material ether extraction was not at first resorted to. Attempts were made to absorb the T.N.T. derivative with charcoal; also to precipitate it with lead and basic lead acetates and to extract it by concentration of the urine at low temperatures. All these methods proved unsatisfactory, as the substance is very unstable in the urine, especially if the reaction becomes alkaline.

The following method was finally resorted to :

The urine was extracted once with ether without previous acidification ; this first ether extract was discarded as it contained nothing that gave a colour reaction with the alcoholic potash. The urine was then acidified with half its bulk of 20 per cent. sulphuric acid and extracted twice with ether. The ether extract was well washed with water and dilute sodium carbonate solution to remove acid and finally dried over anhydrous sodium sulphate. It was noticed that, after washing with sodium carbonate solution, the ether extract now gave a beautiful pure blue colour on treating with alcoholic potash, in contradistinction to the varying red to purple colour previously given. This observation was made use of to modify the Webster test and was found to be a great improvement. It was subsequently independently discovered by Tutin (4).

After drying over anhydrous sodium sulphate, the ether was removed by distillation and the somewhat sticky brown residue was investigated. It was found to be partially soluble in hot alcohol ; the alcohol-soluble part gave only a weak reaction with alcoholic potash while the insoluble part gave a most intense reaction. The residue also reduced to some extent alcoholic silver nitrate on warming on the water bath, giving a silver mirror. This suggested the possibility of a hydroxylamino or related body being present, and on looking up the literature it was found that Cohen and Dakin (5) had prepared 2:6-dinitro-4-hydroxylaminotoluene and 2:6-dinitro-4-toluidine by reducing symmetrical T.N.T. with hydrogen sulphide. They investigated the hydroxylamino compound and found that it was decomposed by boiling with strong hydrochloric acid into a white substance melting at 212° C. and characterized by its very sparing solubility in most organic solvents. They concluded from analysis that this substance was 2:6-dinitro-4-aminotoluene and that the other product of reduction, a reddish brown substance melting at 167° C., was 2:6-dinitro-4-toluidine.

These three substances were prepared from T.N.T. by Cohen and Dakin's method and their behaviour with alcoholic potash investigated. It was found that the hydroxylamino compound gave a violet colour, the 2:4-dinitro-6-aminotoluene a pure blue colour ; the 2:6-dinitro-4-aminotoluene gave no colour.

With these interesting results in view the ether soluble mass obtained from the T.N.T. urines was further investigated. On the supposition that some hydroxylamino compound was present, some of the residue was boiled with concentrated hydrochloric acid and two crystalline bodies were identified: (1) a yellowish brown substance melting at 162° C. soluble in HCl and alcohol, and (2) a white substance difficultly soluble in any solvent but finally crystallized from boiling xylol. Substance (1) gave no colour reaction with alcoholic potash ; substance (2) gave the characteristic blue colour.

About this time a paper from the German chemists Anschütz and Zimmerman (6) appeared. Anschütz and Zimmerman showed that Cohen and Dakin were incorrect regarding their investigation of 2:6-dinitro-4-hydroxylamino compound, which on boiling with concentrated HCl gives rise, not to dinitrotoluidine, but to one dinitroaminotoluene (2:6-dinitro) and 2:6-dinitro-4-azoxytoluene.

Substance (2) obtained from T.N.T. human urine as described above was found to be identical with synthetic 2:6-dinitro-4-azoxytoluene. It had the same melting-point (212° C.); mixed with the synthetic substance its melting-point remained the same. It gave the same colour reaction with alcoholic potash; also its molecular weight as determined by Barger's method was the same as that of synthetic 2:6-dinitro-4-azoxytoluene determined by the same method.

It was naturally assumed that substance (1) would prove to be 2:6-dinitro-4-aminotoluene, but while its melting-point was very near that of the synthetic body (162° C. against 168° C.), the mixed melting-point dropped to 120° C. It was not found possible to identify this substance, though it is highly probable that it is a dinitroaminotoluene. The published work on the dinitroaminotoluenes is by no means satisfactory.

Many attempts were made to isolate the hydroxylamino compound from the urine but without success. The reduction of the alcoholic silver nitrate may not improbably have been due to a trace of something else present.

We were also able to obtain the azoxy compound without boiling the ether soluble mass with concentrated HCl.

Rabbits, cats, and a monkey were given T.N.T. dissolved in olive oil subcutaneously and the urines investigated. The injections were kindly given by Dr. H. H. Dale or Dr. G. A. Wyon. From rabbits' urine, as also from the monkey's, we isolated the azoxy compound, but it is not present in cats' urine. The urine of cats that have been given T.N.T. either by the mouth or under the skin give a negative Webster reaction. The urine, however, on acidification and extraction with ether gives a yellow substance soluble in alkalies and evidently an acid; owing to cats succumbing somewhat readily to T.N.T. it was not possible to obtain sufficient urine to proceed with the investigation.

Rats apparently react still differently, as the urine of these animals after feeding on T.N.T. is of a bright pink colour, although it may be quite acid.

It was stated in Professor Moore's monograph that the hydroxylamino compound excreted in the urine (rabbits', human, and monkeys') conjugated with glycuronic acid, and that this broke down on treatment with acid into free 2:6-dinitro-4-hydroxylaminotoluene and glycuronic acid.

Further work on the urine of rabbits that had been given large doses of T.N.T. failed to substantiate this, and, as will be seen from the above, the only substances that have been definitely isolated are (1) 2:6-dinitro-4-azoxytoluene and (2) a yellowish brown substance melting at 162° C. and not identified, although it probably is a dinitroaminotoluene.

On several occasions the urine of T.N.T. rabbits was found to contain more glycuronic acid than that of control rabbits on the same diet. This was not, however, a constant phenomenon. On one occasion I found in a specimen of rabbits' urine a few milligrams of crystalline yellow nodules. These gave the naphtho-resorcin reaction for glycuronic acid, a violet colour with alcoholic potash,

and left a trace of calcium carbonate on ignition on platinum foil. Unfortunately all attempts to obtain more of this substance failed.

Many attempts were made to isolate from the blood and organs of rabbits dosed with T.N.T. the chromogenic substance, but they were always unsuccessful. Even one hour after an injection of 10 c.c. of 10 per cent. T.N.T. in olive oil, when the chromogenic body was beginning to appear in the urine and there must have been some T.N.T. derivatives circulating in the blood stream, a negative result was given to all tests. The chromogenic body—the azoxy compound —must be formed in the kidney.

It is suggested that T.N.T. is first reduced in the body to a non-chromogenic body, the substance found in the urine melting at 162° C. and not identified, and that the greater bulk of this is transformed by the kidneys into the azoxy body, conjugated with glycuronic acid or glycine and excreted.

This work was done in Dr. H. H. Dale's laboratory under the supervision of Dr. Barger, and it is a great pleasure to me to express my indebtedness to both Dr. Dale and Dr. Barger for very much valuable advice and assistance.

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IV. THE FATE OF T.N.T. IN THE ANIMAL BODY

By H. H. Dale, C.B.E., M.D., F.R.S.

April 29, 1919.

In the Memorandum on T.N.T. poisoning by Dr. Benjamin Moore, an account is given of experiments carried out by Mr. Webster under Dr. Barger's supervision, to determine the form in which T.N.T. leaves the body. Attention was chiefly directed to the chromogen responsible for the urinary reaction discovered by Webster. The chromogenic substance actually isolated was the azoxy compound formed on partial reduction of one of the nitro groups of T.N.T. The fact, however, that most of the extracts from the urine had undergone treatment which would convert the corresponding hydroxylaminodinitroluene into the azoxy compound, led to the suggestion in Dr. Moore's Memorandum that the former was probably the derivative actually excreted. On the other hand, the fact that Webster, as the preceding paper shows, had on occasion obtained the azoxy compound from urine, without treatment such as should have decomposed the hydroxylamino compound, left the matter in some doubt. The point has some significance, in that the hydroxylamino derivative was found by Wyon to be much more poisonous than T.N.T., whereas the azoxy compound is so very insoluble that it can have little significance, at least as an acute poison. It seemed desirable, therefore, to repeat the isolation of the chromogen from urine, under conditions which could not lead to the conversion of the hydroxylamino compound, if present, into the azoxy compound. It will be seen that the result was to identify the chromogen definitely as the azoxy compound. No evidence of the presence of the hydroxylamino compound could be found.

There were other points, of more importance, on which information was not yet available. The chromogen is easily detected in small amounts, and there had been a natural tendency to regard it as the only, or at least the most important, end-product of T.N.T. metabolism in those species in which it appears in the urine. Such an assumption could only be justified strictly by a quantitative determination, showing that a high percentage of the T.N.T. taken into the body reappears in the urine in this form. The fact that in some species (carnivora) no chromogen is found in the urine showed that there were other possible paths for the metabolism of the substance ; and the fact that these species were found to be more susceptible to T.N.T. poisoning than those excreting the chromogen suggested that such alternative paths might be more closely associated with the toxic action. The sensitiveness of these species made it very difficult to follow the fate of T.N.T. in them, since they would not tolerate a dosage sufficient to give a reasonable chance of isolating and identifying the end-products in the urine. It seemed more important

however, to attempt to determine, in a species giving the typical Webster reaction in the urine, what proportion of the T.N.T. given was represented by the chromogen excreted, and whether any of the remainder could be identified in the form of other end-products. The rabbit was chosen as the most convenient animal for the purpose, and its tolerance for very large doses of T.N.T. facilitated the investigation. Full-grown rabbits were used, and the T.N.T. was injected hypodermically as a 4 per cent. solution in warm olive oil. The dose given was 0·4 grm. daily to each rabbit. Accidental circumstances caused occasional intermission of the injections for a day or two, but the urine was collected continuously. It will be seen that several rabbits died during the course; others stood the treatment better. No significant macroscopic changes were noted post-mortem in the organs of those which died. The dosage was deliberately pushed to the limit of tolerance, in order to obtain the maximum concentration of end-products in the urine. The urine was taken from the collecting-cages once or twice daily. Samples which could not be worked up immediately were preserved under a layer of ether for a day or two until they could be treated. The chemical investigation was carried out by Mr. F. Tutin, seconded for the purpose to my department by the Ministry of Munitions. The chemical details which follow have been supplied by Mr. Tutin.

Some preliminary investigations were conducted on the urine of rabbits which had received doses of 0·2 grm. of T.N.T. per diem, but since this urine gave only a relatively slight 'Webster reaction' the work was continued with urine from rabbits which had each received 0·4 grm. T.N.T. daily, and this report embodies chiefly the results obtained from the latter.

The following table indicates the treatment of these rabbits and the quantities of urine obtained:

Day.	*Number of rabbits.*	*T.N.T. administered per rabbit.*	*Volume of urine.*	*Remarks.*
1st	4	0·4 grm.	325 c.c.	
2nd	4	0·4 „	525 c.c.	
3rd	4	0·4 „	870 c.c.	
4th	4	0·4 „	430 c.c.	2 rabbits died.
5th	2	0·4 „		
6th	2	No injection	675 c.c.	
7th	2	No injection		1 rabbit died.
8th	1	0·4 grm.		
9th	1	0·4 „	290 c.c.	
10th	3	0·4 „	420 c.c.	2 fresh rabbits.
11th	3	0·4 „	815 c.c.	
12th	3	0·4 „	935 c.c.	
13th	3	No injection	995 c.c.	
14th	3	0·4 grm.	940 c.c.	
15th	3	0·4 „	970 c.c.	
Amount of T.N.T. injected (deducting for two dead rabbits)				13·2 grm.
Volume of urine investigated				8,190 c.c.

All the batches of urine were slightly alkaline to litmus, and were brown in colour when fresh, but they soon became darker and somewhat blackish on keeping, particularly when exposed to light.

The urine, after filtration through paper pulp, was repeatedly extracted with ether until practically nothing further was removed. It was always found necessary, however, to add a considerable

proportion of alcohol to cause the separation of the emulsions formed, so that the extractions were really effected with an ether-alcohol mixture. This first extract may be termed (A).

The urine was then acidified by the addition of one-tenth of its volume of strong hydrochloric acid, and another similar extraction with ether (+ alcohol) carried out. This second extract may be termed (B), and the doubly extracted residual urine (C).

Examination of (*A*). (Ether extract before acidification.) This ether-alcohol extract did not give any appreciable reaction with ' Webster's test '. It was first washed with successive quantities of water until free from alcohol. The aqueous washings were found to contain some amorphous, brown-coloured material, together with much urea.

The ethereal liquid was then extracted with dilute aqueous sulphuric acid, the acid liquids being subsequently rendered alkaline with sodium carbonate and again extracted with ether. In this way a small amount of an organic base was obtained which was sparingly soluble in water, but readily so in dilute acids. It was, however, entirely amorphous, and the amount was so small that no definite product could be obtained from it.

The ethereal liquid which had been extracted with acid was then washed several times with dilute, aqueous ammonium carbonate, and finally with water. The alkaline extractions removed only some dark-brown tar-like material. The ethereal solution was then dehydrated, and evaporated to dryness. When the residue was dissolved in alcohol and the solution again evaporated to dryness on a water bath, there were left two distinct products which were immiscible ; one, a thick, dark brown, tarry product ; the other, when warm, a clear yellow oil. These were separated by treatment with light petroleum, in which the oil only dissolved. This oil amounted to about 0·2 grm., and readily became partially solid on cooling. On hydrolysis with alcoholic potash it yielded a soap, and, on acidifying the latter, solid fatty acids were obtained. It would appear, therefore, that this fatty oil was not derived from the olive oil in which the T.N.T. had been dissolved prior to injection, but had been excreted as a result of the action of the T.N.T. on the rabbits. No similar product could be obtained from normal rabbit's urine.

The dark brown tarry product from which the oil had been removed by means of petroleum was treated with dry ether, when it was found that a portion of it had become insoluble, and this was removed as a dark brown, amorphous powder. The material, soluble in ether, which was small in amount, was dissolved in a little benzene, and the solution allowed to evaporate slowly. A very small quantity of crystals then slowly formed which were drained on a tile from the thick, dark brown mother liquor. The small amount of impure crystals thus obtained gave a strong ' Webster reaction '. They appeared to consist of a trace of the chromogen which had existed in the urine in the free state. It will be seen later that the chromogen is the azoxy compound.

Examination of (*B*). (Ethereal extract of urine after strong acidification.) The combined ethereal extracts of the acidified urine

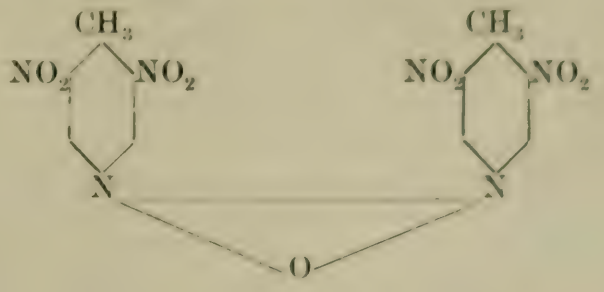
gave a strong ' Webster reaction '. They were washed with water, but this removed only alcohol and a little amorphous colouring material. The washed ethereal liquid was then extracted with aqueous ammonium carbonate when about two-thirds of the material dissolved in it was removed. The alkaline extracts, which were brown in colour, were acidified, and extracted with ether. On removing the solvent a dark-coloured residue was obtained which appeared to consist of organic acids. It was esterified by means of methyl alcohol and dry hydrogen chloride, and the resulting esters isolated. On steam distillation of the latter the greater portion passed over as a colourless oil, which was identified as methyl benzoate. The non-volatile portion consisted only of a small amount of very dark brown tar, from which nothing definite could be isolated. A sample of normal rabbits' urine, when subjected to a treatment identical with that applied to the urine from the T.N.T. rabbits, yielded no benzoic acid. It would appear probable, therefore, that the administration of T.N.T. had prevented the formation of hippuric acid and allowed the benzoic acid to be excreted in the free state. The effect is suggestive of an injurious action on the kidneys, inhibiting the normal synthesis of hippuric acid in those organs.

The ethereal solution, which had been extracted with aqueous ammonium carbonate, was washed, dried, and concentrated to about 200 c.c. On keeping this liquid it slowly deposited a dark brown, amorphous powder, which was removed by filtration, when it was found to be insoluble in all the usual solvents. It dissolved readily, however, in concentrated sulphuric acid, and appeared very similar to the insoluble, dark-coloured products which are formed from T.N.T. by the action of light or of certain bases.

The ethereal filtrate was evaporated to dryness, and then treated with dry ether, when a further amount of the above-mentioned, insoluble, amorphous product separated, and was removed by filtration. These operations of evaporation, re-treatment with dry ether, and filtration, had to be repeated a considerable number of times before the separation of amorphous, insoluble material ceased. This amorphous powder represented eventually about half the original neutral material which had been in the ether extract. It would appear to be derived from the T.N.T., since it deflagrated on heating, but nothing crystalline could be obtained from it.

The final ethereal filtrate from the amorphous powder was treated with animal charcoal, it having been ascertained in previous experiments that the crystalline substances which it contained were not removed from the ether by this treatment. The filtrate was then evaporated completely and the residue dissolved in about 50 c.c. of hot alcohol. On thoroughly cooling this solution a quantity of brown, semi-crystalline solid separated, which appeared almost insoluble in cold alcohol. By further treatment with animal charcoal in benzene solution, and several crystallizations alternately from small quantities of the latter solvent and large volumes of hot alcohol, it was obtained in small, colourless leaflets, and was identified as the azoxy compound, being identical with the compound formed by heating 4-hydroxylamino-2 : 6-dinitrotoluene with concentrated

hydrochloric acid. The total amount isolated was 0·40 grm.—3 per cent. of the total amount of T.N.T. administered. It will be noted that this compound was extracted without subjecting the extract to any treatment which could lead to its formation from a pre-existing hydroxylamino compound.



The original alcoholic filtrate from the crude azoxy-compound was evaporated until it formed a thin, very dark brown syrup. On keeping the latter, crystals slowly commenced to form, but their separation was not completed until after several weeks' standing. The thick, dark-coloured syrup containing the crystals was then placed on a porous tile and allowed to drain in an atmosphere saturated with alcohol vapour, when a yellow, crystalline solid was left on the tile in a fairly clean condition. After re-crystallization from a small volume of absolute alcohol this substance melted at 154–155°, but after several crystallizations, alternately from small volumes of absolute alcohol and hot benzene, the melting-point was raised to 171°. The total amount obtained from the quantity of urine under treatment was 0·52 grm.—4 per cent. of the total dosage of T.N.T.

A very small quantity of another substance, of a much lighter colour, was contained in the mother liquors, but this could not be isolated. The total amount of crystalline derivatives of T.N.T. isolated in a state of purity therefore amounts to almost exactly 7 per cent. of the weight of the T.N.T. administered to the rabbits.

The identification of this yellow compound, representing a larger proportion of the T.N.T. administered than the Webster chromogen, was obviously a matter of some interest. It is probably identical with the substance obtained by Webster, which he also found to be very similar to but not identical with the only known amino-dinitrotoluene (see p. 51). Unfortunately its examination has been attended with difficulties which have prevented its identification with complete certainty. Its properties suggested that it might represent a further stage of reduction than the azoxy-compound, one nitro-group of the T.N.T. being fully reduced to an amino-group, thus forming an amino-dinitrotoluene. Two such compounds are possible as products from the symmetrical T.N.T.—2-amino-4:6-dinitrotoluene, and 4-amino-2:6-dinitrotoluene. These both occur in the literature of the subject, having been described by Holleman and Böseken. According to these authors the 2-amino-compound melts at 155°, and this was the melting-point of the compound from the rabbits' urine when first obtained. On further purification, however, the melting-point of the product

from urine rose to 171°, which is that of 4-amino compound, as determined by Holleman and Böseken. A sample of the latter was prepared, and was found to be very similar in its solubility and general properties to the product from urine, but the melting-point of the two, when mixed, fell much below the temperature of 171°, at which both individually melted when pure.

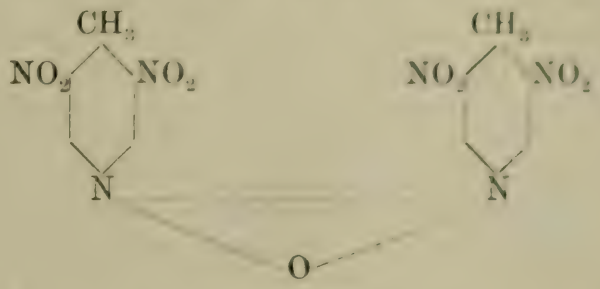
So far the evidence would seem to exclude either formula as that of the product from urine, but a further investigation of the second material obtained by Holleman and Böseken's method, and described by them as the 2-amino-dinitrotoluene, revealed the fact that it was really an impure sample of the 4-amino compound, the melting-point rising gradually with repeated purifications until it reached 171°. All attempts to prepare the genuine 2-amino compound by synthesis failed, so that the possibility remained open that the product from urine had this structure. So far only the 4-amino structure was definitely excluded.

An accurate analysis would have greatly strengthened the attribution of such a constitution to this body, but unfortunately its explosive properties foiled repeated attempts to obtain trustworthy figures by combustion. Determinations of the molecular weight, on the other hand, made by Barger's microscopic method, and by the cryoscopic method in glacial acetic acid, were quite consistent with such a formula. The values obtained by the two methods were 200 and 209 respectively, that theoretically required for an amino-dinitrotoluene being 197.

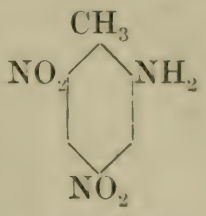
As another possible method of identification, the removal of the amino group, if present, suggested itself; the dinitrotoluene resulting should have been identifiable without difficulty. The conditions were first determined under which this transformation could best be effected with the synthetic 4-amino compound, and a good yield of 2:6-dinitrotoluene was obtained from this. The 5-amino-2:4-dinitrotoluene (prepared from γ-T.N.T.) was treated by the same method and with difficulty yielded a moderate amount of 2:4 dinitrotoluene, with a small amount of a substance having the properties of a dinitrophenol. The 3-amino-2:4-dinitrotoluene (obtained from β-T.N.T.) was quite unaffected by the same treatment. It was evident, therefore, that the method, consisting of diazotizing and pouring the reaction product into boiling absolute alcohol, though perfectly successful with the 4-amino compound, was varyingly successful with those having the amino group in other positions. On treating the product from urine, the diazotization appeared to proceed normally, but on working up the product obtained by pouring into alcohol, the main portion was found to consist of a substance having the properties of a dinitrophenol. It formed hard yellow crystals, which darkened on exposure to light, and dissolved in aqueous sodium carbonate to a deep yellow liquid. A small amount of neutral material was also formed, but contained at most a trace of a dinitrotoluene. The greater part of this small neutral residue consisted of a nearly colourless crystalline compound (m.p. 204°), which gave a deep, but transient red colour with alcoholic potash.

Since neither 2-aminodinitrotoluene itself, nor the corresponding

phenol, with the hydroxy group replacing the amino group, was known or could be prepared, the identification of the yellow substance from the urine remained incomplete. At the same time there was established a strong probability in favour of its being the 2-amino compound. If it is so, it would appear that T.N.T. in the rabbit can undergo reduction in one of two positions. If the NO_2 group in the *para* position relative to the methyl is reduced, the reduction proceeds only as far as the formation of the azoxy compound



This is excreted in the urine as a water-soluble complex, probably, as suggested by Webster, with glycuronic acid. It is only when this is decomposed by the hydrolytic action caused by acidifying the urine, that the free azoxy compound can be extracted with ether and give the characteristic colour reaction of the Webster test. If, on the other hand, reduction takes place on a nitro group in the *ortho*-position relative to the methyl group, the reduction is apparently complete, producing 2-amino-4 : 6-dinitrotoluene



which gives no colour-reaction with alcoholic potash, but is excreted, like the azoxy compound, as a complex which must be hydrolysed before extraction with ether becomes effective.

A somewhat larger proportion of the T.N.T. leaves the body as the (?) aminodinitrotoluene than as the azoxy compound; but the quantities obtained pure of the two together only amounted to 7 per cent. of the T.N.T. administered. The fact that these compounds are not very stable makes it certain that the true yield was somewhat greater; but even if it be assumed that the whole of the amorphous material, removed from the ethereal extract (B) in the process of purification, represented products of decomposition of these two bodies, this would do little more than double the combined yield. There would still be some 85 per cent. of the T.N.T. administered to be accounted for in other ways. Without doubt a large proportion of it was excreted in the form of highly coloured and unstable compounds, which on any attempt at isolation formed tarry and resinous substances from which nothing could be isolated in a state of chemical purity. These dark tars certainly represent the greater part of the T.N.T. absorbed, and their presence suggested

that most of the T.N.T. is changed in the body into products less stable and more prone to oxidative decomposition than either of the two derivatives isolated—the azoxy compound or the (probable) aminodinitrotoluene. The presence of such more readily oxidized derivatives was suggested also by the red staining of animal's fur by the urine—a reaction given by neither of the identified derivatives —and by the darkening of the urine on keeping. The derivatives with such properties most likely to be formed in the body from T.N.T. are those in which two nitro groups are reduced—diamino-mononitrotoluenes. Such compounds are known to be extremely unstable ; attempts by chemists to prepare them synthetically have been frustrated by the formation of tarry decomposition products. If they occurred in the urine, no ordinary method of isolation would be likely to succeed. It seemed possible, however, that they might be obtained as derivatives, if the sensitive amino groups were protected, as by acetylation. If such compounds were present, the basic properties due to the presence of two amino groups would probably prevent their extraction from the acidulated urine by ether, and they should remain in the residual urine (C).

Examination of (C). Successive quantities of this residual urine were evaporated to dryness *in vacuo* and treated with acetic-anhydride in the presence of pyridine. The resulting product was extracted with ether, but in each case only a trace of crystalline substance could be obtained, which was much too small to permit of any attempt at identification.

The conversion of T.N.T. in the body into more highly reduced compounds remains, therefore, nothing more than a suggestive possibility. If such compounds were formed their presence might have much significance for the toxic action. With such further reduction the product would approach more nearly the structure of toluylenediamine, the toxic action of which on the blood and the liver is one of the classical observations of pharmacology. The only definitely isolated end-products, however, are still the azoxy compound, which is the Webster chromogen, and another crystalline substance, which is probably, but not certainly, 2-amino-4 : 6-dinitrotoluene.

Some considerations as to the significance of the Webster test are suggested by these observations. It has been recommended and used as a signal indicating danger of T.N.T. poisoning. It is a definite indication that T.N.T. in recognizable quantity is being absorbed, and so far its use as an index of danger is justified. If the reaction is prevalent and strongly marked in the urines of workers in a factory, it may serve as an indication for increased precaution against absorption of T.N.T. On the other hand, its appearance in the urine of a member of a group known to be exposed to T.N.T. absorption cannot be properly regarded as an index of individual danger of poisoning. The azoxy compound responsible for the colour-reaction is very insoluble in all kinds of solvents—so insoluble that no satisfactory method of testing its toxicity has been devised. This property alone makes it probable that it is less poisonous than T.N.T. itself. Its appearance in the urine is an index that it is being properly dealt with by the body, by condensation with

glycuronic acid and excretion. It may be suggested that the *absence* of this reaction from the urine of a patient who is known to be absorbing T.N.T. would be the really ominous sign. The evidence is so incomplete that no definite statement is justified; but the weight of probability seems certainly to be in favour of the view that the danger of serious intoxication will increase with the tendency to the formation in the body of more highly reduced derivatives of T.N.T., and that excretion as the azoxy compound represents a stoppage of reduction at the safest point. In appraising the significance of the Webster reaction in an individual case, its bad significance as an index of T.N.T. absorption must be balanced against its probably good significance as an indication that the body is dealing with what is absorbed in the safest way. It must be added that no data are available to show how far the conclusions, based on experiments with massively dosed rabbits, as to the small proportion of the T.N.T. excreted in this form, can be applied to men absorbing relatively small amounts.

V. SUMMARY OF AN EXPERIMENTAL INVESTIGATION INTO T.N.T. POISONING

By P. N. Panton, M.B., B.C., Cantab., *Clinical Pathologist to the London Hospital*, and Harold E. Bates, M.D., Baltimore, U.S.A.

In the course of the investigation of cases of poisoning among T.N.T. workers certain problems called for experimental work on animals.

First as to the exact nature of the toxic substance. T.N.T. itself was originally considered to be non-toxic, and attention was directed to impurities and decomposition products of T.N.T.

One of the first possible sources of poisoning to which attention was called was the inhalation of fumes given off by T.N.T. Nitrous fumes in sufficient amount to be detected by the sense of smell are given off by T.N.T. Dr. Benjamin Moore (*Proc. Roy. Soc. Med.*, Jan. 1917) pointed out that workers in processes which involve the inhalation of much larger quantities of nitrous fumes than in the handling of T.N.T. are not affected by either jaundice or anaemia. In this connexion one of us with Dr. Vaidya exposed rabbits and white rats to nitrous fumes in a fume chamber over long periods of time. The percentage of NO in the chamber was far greater than ever could occur in any T.N.T. process. It was found that the animals even with small quantities of gas showed cyanosis, and gave a positive Haldane's test for methaemoglobinaemia. Repeated blood examinations were made and in no case was there any anaemia. The animals showed no jaundice and blood serum taken at intervals showed no bile. At post-mortem examination no changes were found in the livers comparable to those in cases of T.N.T. poisoning.

The following experiments were performed.

A rabbit weighing 4 lb. 4 oz. was placed in a chamber of concentrated NO fumes for two hours at a time. It had a total of thirty hours in the chamber, the experiment lasting over a period of three months. Five blood examinations were made, there was no appreciable change in the red-cell count, haemoglobin percentage, white-cell count, or differential. No bile was present in the blood serum. The animal was cyanosed after each exposure to the fumes, and Haldane's test for methaemoglobinaemia was positive. At post-mortem examination the liver, kidneys, spleen, and bone-marrow were normal. The lungs were deeply congested and microscopical examination of sections showed areas where the alveoli were filled with blood, epithelial cells, and a fair number of polymorphonuclear cells.

A second rabbit, weighing 5 lb. 4 oz. was placed in the NO fume chamber for two-hour periods nearly every day for nine weeks, the experiment extending over a period of three months. Five blood examinations were made and no appreciable change in the counts was found. No jaundice was seen, and no bile was found in the blood serum. Cyanosis was present after each exposure and Haldane's test was strongly positive. Post-mortem examination showed the liver, kidney, spleen, and bone-marrow normal. The lungs showed areas of congestion, and the microscopical picture was similar to that of the first rabbit.

Two rats were also treated in a similar manner and showed no blood changes and the viscera were normal at post-mortem examination.

The only substance in high explosives other than T.N.T. which is used in large quantities is ammonium nitrate, which is mixed with T.N.T. up to a proportion as high as 80 per cent. Ammonium nitrate, however, is believed to be quite harmless.

A rabbit weighing 4 lb. 7 oz. was given by hypodermic injection 5 grm. ammonium nitrate dissolved in distilled water at each injection. Fifteen doses in all were given or a total of 30 grm. The experiment lasted two and a half months. Three blood examinations were made. There was no change in the red-cell count, white-cell count, or differential. There was no jaundice and no bile in the blood serum. At post-mortem examination the organs were normal throughout.

Leaving out of account certain substances, such as tetranitromethane, which occurs in very small quantities in commercial T.N.T. and the toxic action of which has been investigated by others, the obvious conclusion is that T.N.T. itself is the toxic substance. The direct proof of this is, however, difficult to obtain. The main difficulty has been the original failure to reproduce the major lesions of T.N.T. poisoning in animals, and the more recent experiments have been only partially successful. In the first series of these experiments carried out by one of us and Dr. Vaidya commercial T.N.T. was used. Five rabbits were injected with varying amounts of T.N.T. dissolved in toluol. Repeated blood examinations were made and post-mortem notes were taken. In none of these experiments were the major lesions of T.N.T. poisoning reproduced.

We will give the details of these very briefly.

A rabbit weighing 4 lb. was given by hypodermic injection 50 mg. of T.N.T. daily with certain intervals. A total of 1·15 grm. of T.N.T. was given over a period of two months. Six blood examinations were made. There was no marked change in the blood picture, no jaundice and no bile in the blood serum. Because of a subcutaneous abscess the animal was killed. At the post-mortem the organs were normal throughout.

A rabbit weighing 3 lb. was given a total of 1·7 grm. of T.N.T. in doses ranging from 50 mg. up to 500 mg., over a period of two months. Six blood examinations were made with no appreciable change in the counts. No jaundice was present and no bile in the serum. Organs were normal throughout at post-mortem.

A rabbit of 3¾ lb. was given a total of 0·9 grm. of T.N.T. in 100 mg. doses over a period of three weeks. The animal developed an abscess at the site of injection and was killed. Four blood examinations were made with no change in the counts. There was no jaundice nor bile in the blood serum. The organs were normal on post-mortem examination.

A rabbit of 6 lb. was given one dose of 500 mg. T.N.T. and died ten days later. The organs were normal at post-mortem examination. One blood count only was done, which showed nothing abnormal.

In attempting to explain the fact that such a small percentage of T.N.T. workers suffer from the poisonous effects, it was suggested that the poisoning might arise by a process of sensitization, by which a worker receiving small doses of the poison at intervals might render himself sensitive to a subsequent dose. In this connexion it was attempted to sensitize a rabbit by alternating small and large doses. This, however, failed to have any other effect than the regular mode of administration.

A rabbit weighing 3 lb. 10 oz. was given a total of 2·4 grm. of T.N.T. This was given in alternating doses of 50 mg. and 500 mg. (with one dose of

750 mg.) at intervals of ten days. Three blood examinations were made, with no change in the blood picture. There was no jaundice and no bile in the blood serum. The organs were normal throughout at post-mortem.

The animals used in the earlier experiments were rabbits, guinea-pigs, and rats. That the metabolism of the drug varies in different species was proved by Mr. Webster and Dr. Wyon working under the auspices of Dr. Dale and Dr. Barger (*loc. cit.*). It was subsequently shown that cats were more sensitive to T.N.T. than rabbits. All of our later experiments have been made upon cats, and while the results have not been particularly encouraging, it has been possible to reproduce some of the liver changes, with typical central necrosis of the liver lobules though never the massive necrosis found in the human cases, and one typical case of aplastic anaemia was developed.

A possible explanation of the small proportion of workers affected, and the difficulty of reproducing the lesions in animals was that of variation in the composition of the drug itself. Attention was therefore directed to the different toxic effects of the three T.N.T. isomers. The great bulk of the powder consists of the symmetrical or α-T.N.T. The β- and γ-T.N.T.s vary in amount in different samples from a mere trace to 4 per cent. in an exceptional sample analysed by Mr. F. Tutin. We therefore made several experiments to determine the effect of the three T.N.T. isomers with the advice and assistance of Dr. Dale.

Another suggestion, made by Dr. Laidlaw, was that the introduction of colon bacilli in addition to the T.N.T. might succeed in reproducing the liver changes. This also we tried.

We wish to call attention to the fact that the number of experiments is wholly inadequate for conclusive results, but the amount of work required for even a small number of experiments was more than could be accomplished in the time at our disposal.

In all thirteen cats were used. As the T.N.T. was given dissolved in toluol, one cat was given toluol alone as a control.

It was particularly attempted to reproduce aplastic anaemia or the liver changes or both. The method of administering the drug was the same in all cases. The different T.N.T. isomers were dissolved in toluol and given by hypodermic injection beneath the skin of the lumbar region.

The general effect of the drug may be considered first. Where the drug was given in small repeated doses over a considerable period, loss of weight was a constant and well-marked feature. The animals became drowsy, refused their food, lost weight rapidly and died. Frequent intermissions were therefore necessary. Especially was this the case in cats treated with α-T.N.T.; although the lethal dose for the cat of T.N.T. β or γ is smaller than that of α, repeated small doses of β and γ seem to be better tolerated than in the case of α.

Cyanosis was noticeable in the early stages of chronic poisoning, but this was later obscured by the yellow colour which developed.

Jaundice, as evidenced by a yellow colour of the skin and mucous membrane, was constantly present, in varying degree. The skin and subcutaneous tissue at post-mortem were in some cases deeply yellow. This yellow colour, however, as pointed out by Dr. Dale,

was not due to the presence of bile pigments in the blood, but to a combination of the T.N.T. with the body proteins producing a yellow reaction. The blood serum was deeply yellow in all animals treated with β- and γ-T.N.T. and was not noted in any of the cats which were given pure a-T.N.T.

Local necroses at the site of injection were common. These necroses were usually sterile, but occasionally an extensive abscess developed and the animal had to be killed.

In estimating the effects of these drugs upon the histology of the organs we availed ourselves of the kind assistance of Dr. H. M. Turnbull. In two instances Dr. Turnbull made the complete post-mortem examinations and in other cases examined the sections for us. Dr. Turnbull's reports and remarks are placed in inverted commas and initialled.

The effect of the drug on the *condition of the blood.* In eight cats periodic blood examinations were made, and in seven no specific blood changes were produced. The eighth case is of considerable interest.

A cat was given small doses of γ-T.N.T. by Dr. Dale over a period of from nine to ten weeks. 50 mg. given daily with remissions. The first blood examination showed:

Red cells	6,750,000 per c.mm.
White cells	22,800 per c.mm.

Differential count:

Polynuclear neutrophils	89·5%
Small lymphocytes	5·0
Large lymphocytes	0·5
Large hyaline	5·0

One normoblast was seen among 200 leucocytes. There was some polychromatophilia. This is a comparatively normal blood count for a cat.

Six days later the cat was very ill, and the blood examination at that time showed:

Haemoglobin	18%
Red cells	1,937,500 per c.mm.
White cells	600 per c.mm.

Differential count:

Polynuclear neutrophils	8%
Small lymphocytes	58
Large lymphocytes	18
Large hyaline	16

Sixteen normoblasts were seen in counting 100 leucocytes. Occasional cells showed polychromatophilia.

The cat was then killed and a post-mortem examination was made. Wasting was marked. The liver, spleen, and intestines were covered with lymph. The liver was definitely enlarged weighing 124 grm., the cat weighing 2,100 grm. A liver from a normal cat weighed 82 grm., the cat weighing 3 kilos.

'In the liver there were grey cellular thrombi in the hepatic veins. Close to the portal sheaths were some areas of haemorrhage, sometimes containing necrosed hepatic cells; these may have been the result of the venous thrombosis. Leucocytosis was very conspicuous throughout the liver. The large veins of the spleen were also thrombosed. The pulp was hyperplastic and engorged. There was no proof of myeloid transformation.'—H.M.T.

The shaft of the femur was well filled with red marrow. Films from this showed almost entirely mononuclear cells of lymphoid and endothelial types, scanty eosinophiles mainly mononuclear, numerous normoblasts and occasional megaloblasts. No phagocytosis was seen.

The changes in the blood as shown by the rapid loss of red cells without marked change in their morphology, and the extreme leucopenia together with the almost total disappearance of the granular marrow cells are identical with the changes in the human cases.

Another cat, which was injected with γ-T.N.T. in which the blood was periodically examined over a period of four to five months, showed no blood changes whatever.

The Liver changes.

Brief details of the experiments.

Cat No. I. 50 mg. doses of α-T.N.T. by subcutaneous injection over a period of thirty-nine days.

Total amount given —350 mg.
Weight on March 12—3,980 grm. ; on April 9—1,040 grm.

Blood examinations.	*Mar.* 1.	*Mar.* 11.	*Mar.* 19.
Haemoglobin . . .		25%	85%
Red cells	8,725,000	10,937,000	8,875,000
White cells. . . .	21,640	32,000	28,000
Differential :			
Polynuclear neutrophils .	83·5%	75·5%	
Coarsely granular oxyphils .	5·5	5	
Small lymphocytes . .	7	13	
Large lymphocytes . .	2	4	
Large hyaline . . .	2	2·5	

Cat died. Post-mortem showed slight cyanosis. An extensive subcutaneous abscess. Viscera were entirely normal in appearance. Sections of the kidney were normal with the normal amount of fat. Sections of *the liver* : ' The central zones of the hepatic lobules were slightly fatty. Leucocytic infiltration of portal sheaths was conspicuous. There was a focal necrosis infiltrated by leucocytes close to a hepatic vein.'—H.M.T.

Cat No. II. 50 mg. doses of α-T.N.T. for twelve days. Total amount 300 mg. Only one blood examination was made which was fairly normal. The cat developed an extensive abscess and was killed. At post-mortem examination there was found to be an extensive subcutaneous slough over the back extending to the abdominal wall. The viscera were normal in appearance. ' There was a hyperplasia of the splenic pulp in which megakaryocytes were present. There was no leucocytosis in the capillaries of the liver.'—H.M.T.

Cat No. III was given a total of 3,125 grm. β-T.N.T., beginning with 50 mg., a dose gradually increased to 125 mg. Over a period of three and a half months. It steadily decreased in weight from 5,340 grm. on March 12 to 1,930 grm. on July 7. The blood serum examined on several occasions was distinctly yellow. Seven blood examinations were made between March 11 and July 7. These counts varied slightly from time to time but within normal limits. The last count before death showed a distinct rise in the total number of white cells from an average about 12,000 to 65,000, and the differential count showed an increase in polynuclear neutrophils from an average of 75 to 97 per cent.

The cat died and post-mortem examination showed wasting. The skin and subcutaneous tissue were distinctly yellow. The liver was large and pale with a yellowish tinge, the lobules were well marked, with light centres, and there were what appeared to be numerous small dilated capillaries on the surface. Kidneys were normal. The spleen was large, firm, and somewhat nodular. Nodules were present in the lung.

Microscopical examination of sections : ' The hepatic cells were fatty, especially in the peripheral zones of the lobules. The only pathological conditions of which I could be certain were a great engorgement with dilatation of many hepatic veins and their tributaries, and a striking number of large mononuclear cells, probably large mononuclear leucocytes, within these vessels. There was con-

siderable hyperplasia of the cells of the splenic pulp, amongst which polymorphonuclear leucocytes were numerous, but myeloid transformation was not detectable. The lung contained parasites, apparently nematodes developing from morulae.'—H.M.T.

Cat No. IV was given 50 mg. β-T.N.T. a day, increased to 100 mg. during the last week for 10 weeks, at the end of that time one blood examination was made, which was entirely normal. The cat died suddenly. Post-mortem examination showed many adhesions about the abdominal viscera, the liver being covered by organizing lymph. About the spleen and pancreas was fairly dense new connective tissue.

On microscopical examination, the liver which weighed 84 grm. showed 'considerable dissociation of the hepatic cells but no degeneration. The dissociation was apparently due to mechanical injury after death. The splenic pulp contained few cells. There was a little recent broncho-pneumonia of the lungs.' —H.M.T.

Cat No. V was given one dose of 250 mg. β-T.N.T. No blood examination was made. Post-mortem: 'Both liver and kidney were engorged. The cells of the central and intermediate zones of the hepatic lobules were fatty. There was a little infiltration of the portal sheaths. There was a vesicular degeneration of the hepatic cells immediately round the portal sheaths.'—H.M.T.

Cat No. VI was given a total of 3·4 grm. of γ-T.N.T. over a period of three and a half months. It decreased in weight from 5,340 grm. on March 12 to 3,000 grm. on July 10. Seven blood examinations were made, all of which were comparatively normal. At post-mortem it showed marked yellow staining of the skin and subcutaneous tissue. Liver was large, dark red, with the lobulation well marked on the surface with blood-vessels forming the periphery of the lobules and light yellow centres with a pin-point red dot in the centre. Kidneys and spleen were of normal appearance. Nodules were present in the lungs.

Sections. 'There was extreme fatty infiltration of the liver. There was no hyperplasia of the splenic pulp. Parasites similar to those in Cat III were found developing in the lung.'—H.M.T.

A kitten which weighed 1,075 grm. was given by Dr. Dale a total of 1.345 mg. of γ-T.N.T. At the time of its death it weighed 650 grm. At post-mortem examination the gall-bladder was found full of pus.

Sections. 'The hepatic cells were dissociated, rounded, sharply stained and free from degeneration. This may represent replacement by regeneration of gradually destroyed cells. I could, however, find no karyokinetic figures. There were several focal necroses infiltrated by leucocytes.'—H.M.T.

Two other kittens were also treated but developed no changes.

In reference to the histological specimens considered above, Dr. Turnbull made the following remarks: 'In the sections from this series of experimental cats I have, therefore, not been able to detect lesions of significance. Focal necroses were present in the liver in three cases, but these necroses resembled those found so frequently at necropsy in man and cannot be taken as examples of the hepatic lesions found in trinitrotoluene workers. In man they are associated with various pathological conditions of the intestinal tract, for instance catarrhal inflammation. The staining of the sections did not permit identification of myeloid transformation, except by the recognition of megakaryocytes. In one case only (Cat No. II) were megakaryocytes present in the spleen, indicating a myeloid reaction such as was found in the two cases to be subsequently described (Cats VII and VIII).'

Cat No. VII was given a total of 2·2 grm. of α-T.N.T. in doses beginning with 50 mg., increased gradually to 200 mg. In addition seven intravenous injections of 2,000 million dead *B. coli* each were given. The experiment lasted seven months, at the conclusion of which the animal died suddenly. The animal lost some weight from 1,850 grm. on May 10 to 1,540 grm. on November 29. Three blood examinations were made, all of which were fairly normal.

On the post-mortem examination Dr. Turnbull made the following report:

'The *liver* weighed 90 grammes. A lobular pattern was shown by red dots separated by a coffee-brown reticulum. In sections stained with Sudan no fat was revealed. There was no evidence of degeneration or disturbance of the normal pattern. The Prussian blue reaction showed numerous Kupffer endothelial cells loaded with iron granules, but demonstrated no iron within the columns of hepatic cells. Certain yellow and grey cylinders seen in the upper border of the left lobes, and suspected at the naked-eye examination to be parasitic, proved to be large hepatic veins distended by thrombi. There was no leucocytosis in the blood of this or of any of the other organs. The *myocardium* (heart, 10 grammes) was free from fatty degeneration. The *kidneys* (together 25 grammes) showed great fatty infiltration of the first convoluted tubules. In the *spleen* (2·5 grammes) the pulp was scanty. A few phagocytes contained ingested red corpuscles, and a very few cells contained granules of iron. Leucocytes were few in the pulp. Several megakaryocytes were present in the organ, one being within an intra-trabecular vein, but no other proof of myeloid transformation was found in sections treated with Jenner's stain. The *marrow of the femur* was mahogany red throughout. The microscope showed small patches of lipomatous marrow, and isolated fat cells scattered through the haemopoietic elements. In the latter megakaryocytes were abundant, myeloblasts were in excess of myelocytes and there were numerous foci of erythroblastic activity. In the *lungs* slight collapse and catarrhal pneumonia in a few subcapsular alveoli were revealed by the microscope. The bronchi and bronchioles appeared normal. Several megakaryocytes were present in capillaries. In the *suprarenal* bodies no abnormality was detected. The *pancreas* weighed 6 grammes. In the subcutaneous tissue of the back were two drachms of pale yellow creamy fluid (injections) in films of which no pus cells nor organisms were found. The inner surface of the skin was tinted yellowish-brown, and the saline-formaldehyde in which the portions of the tissue were fixed was stained next morning the same colour.'

Cat No. VIII was given in all 2 grm. *a*-T.N.T., beginning with 50 mg. doses inc sed to 200 mg. Also six subcutaneous injections of 2,000 million dead *B. coli* each were given. The experiment lasted six months. Loss of weight was from 2,240 grm. on June 7 to 1,490 grm. on December 9, when death occurred. Three blood examinations were made, all of which were normal.

Post-mortem report by Dr. Turnbull:

'The *liver* weighed 70 grammes. There was a lobular pattern, small polygonal yellow areas being separated by reddish brown lines. Microscopic examination showed that in the central half to two-thirds of the lobules, and frequently in strands uniting the centres of neighbouring lobules through points equidistant from adjacent portal systems, the hepatic cells were swollen, and often dissociated. Their cytoplasm was relatively faintly stained and eosinophil; it was usually vacuolated, rendered foam-like by numerous vacuoles or occupied by a single large vacuole; occasionally it was hyaline. In Sudan preparations the vacuoles were shown to contain fat. The majority of the nuclei were absent; those that remained were either distorted, or lobed, or pyknotic, or, less commonly, broken into beads (karyorrhexis) or very faintly stained (karyolysis). Into these degenerate and necrosed cell columns there was a slight emigration of polymorphonuclear leucocytes. The Prussian blue reaction with steaming hydrochloric acid revealed abundant iron within the centres of the great majority of the hepatic columns in the healthy peripheral zones, and also in many of the degenerate and necrosed cells of the central zones. Iron was also present in, relatively few, endothelial cells of Kupffer. There was a distinct leucocytosis in the blood throughout the organ, but more conspicuous in the centres of the lobules. White, cellular thrombi were present in many portal veins, hepatic arteries, and hepatic veins. The *myocardium* (heart: 14 grammes) was free from fatty degeneration. The vessels contained a relatively large number of white cells. In a large white *thrombus* resting upon the endocardial surface of the left ventricle the majority of the cells were neutrophil leucocytes, but a few myeloblasts and myelocytes were found (Jenner's stain). In the *kidneys* (together 24 grammes) the first convoluted tubules were loaded with fat, but there were few degenerate nuclei, the condition being, apparently, a fatty infiltration rather than a fatty degeneration. In many ascending limbs of the loops of Henle, however, there were albuminous degeneration and necrosis, whilst there were

many hyaline and granular casts, especially in the descending limbs of the loops of Henle. Thrombi, containing many white cells, were present in several veins. The *spleen* (10 grammes) contrasted sharply with that of the first case. The increase in size and weight was due to a great cellularity of the pulp. The pulp contained a very large number of myeloblasts, many containing karyokinetic figures, a large number of leucocytes, many megakaryocytes, and a considerable number of erythroblasts; myelocytes were, however, very scanty. Many myeloblasts were present in large veins. Many phagocytes contained red corpuscles, and there was a very large number of phagocytes containing coarse granules of iron. The *marrow* of the femur was soft, moist, and a very pale pink. It contained no areas of lipomatous tissue, and only a very few, isolated fat cells. It contained numerous megakaryocytes. Myeloblasts were in great preponderance over all other cells; erythroblastic activity was less than in the previous case, and myelocytes were fewer. In the *lungs* were present catarrhal desquamation in a few subpleural alveoli, and albuminous exudate in one section. The capillaries contained many megakaryocytes, and there was one large megakaryocyte in an arteriole. In the *suprarenal* bodies no abnormality was detected. The *pancreas* weighed 5 grammes. The inner surface of the skin was white.'

Remarks by Dr. Turnbull on the two preceding cases:

'I have, unfortunately, no experience of the normal histology of the cat. That it varies in some respects from the human is shown by the condition of the kidneys in all the cases submitted to me for examination. In the cells of the first convoluted tubules of the kidneys an abundant deposit of fat was unaccompanied by evidence of a corresponding degree of degeneration of the nuclei. Fatty infiltration of the kidney, thus, appears to occur in the cat, though not in man. Conditions such as necrosis can only be regarded as abnormal. Changes in the blood-forming organs found in certain cases only, or in greater degree in certain cases, can also be regarded as abnormal, provided the animals compared are of approximately similar age. Without, however, experience of the reactions of the tissues of the cat, it may be erroneous to ascribe to such changes the same significance as when they occur in man.'

'With these reservations, it may be said that in Cat No. VIII the following abnormalities were present: a fatty degeneration and necrosis of the central zones of the hepatic lobules; haemosiderosis of the liver; parenchymatous degeneration of the kidneys; a general leucocytosis and myelaemia; a conspicuous myeloid, chiefly myeloblastic transformation of the splenic pulp; leucocytosis of the spleen; great erythrocytic phagocytosis in the spleen; increased activity of the haemopoietic marrow, in which myeloblastic activity greatly preponderated.'

'There were, therefore, two main lesions—a central degeneration with necrosis of the hepatic lobules, and a disturbance of the blood-forming organs. The degeneration and necrosis of the liver might have been due to severe anaemia, and the myeloblastic activity of the marrow and accessory blood-forming organs might correspond to that which is found in pernicious anaemia in man. Evidence of excessive destruction of red corpuscles was given in the spleen by the phagocytosis of erythrocytes and the great accumulation of coarse granules of iron derived from ingested corpuscles, and in the liver by the extensive haemosiderosis of the hepatic columns. But, severe anaemia appears to be excluded by the absence of fatty degeneration of the myocardium, and by the relatively slight part played by erythroblastic activity in the changes in the blood-forming

organs. In all severe anaemias in man erythroblastic activity is conspicuous ; even in aplastic anaemia, such haemopoietic marrow as is present is almost entirely erythroblastic.'

' The changes in the blood-forming organs resembled those resulting in response to an excessive call for leucocytes, much more closely than those occurring in anaemia. It would appear, therefore, that the disturbance of blood-formation was not essentially anaemic, and was not the cause of the hepatic necrosis. More probably the disturbance of blood formation was instigated directly or indirectly, by agents which caused necrosis in the liver.'

' The tissues in Cat No. VII showed only slight abnormalities when judged by the standard of normal human tissues. They serve, therefore, at any rate as a control to those of Cat No. VIII. The abnormalities were : evidence of myeloid transformation of organs afforded by the presence of megakaryocytes in the splenic pulp and in the vessels of the lung ; abundant iron in the endothelial cells of the liver. If these are not normal occurrences in cats, they indicate a slight degree of the disturbance of the blood-forming organs which was found in Cat No. VIII.'

Cat No. IX was given 13 c.c. of toluol in 5 c.c. doses over a period of three months. This was done as a control in order to note the effect of toluol as, in all the experiments, the T.N.T. was given dissolved in toluol. There was some loss of weight from 1,630 grm. on May 10 to 1,230 grm. on July 9. Two blood examinations were made, both of which were normal. The blood serum was clear white. The post-mortem showed nothing abnormal to the naked eye.

Sections showed : ' The hepatic cells contained much fat, the cells in the central zones containing more than those in the peripheries of the lobules. There was, however, very little nuclear degeneration. A few portal systems were infiltrated. There was one small, focal area of necrosis ; it was partly haemorrhagic and was bordered by a zone of leucocytic infiltration. The splenic pulp was not hyperplastic and contained no megakaryocytes.'

Résumé

Rabbits and rats exposed to NO vapour developed cyanosis and methaemoglobinaemia but none of the major changes of T.N.T. poisoning.

Ammonium nitrate given hypodermically to a rabbit over a period of ten weeks had no effect.

Five rabbits injected hypodermically with T.N.T. dissolved in toluol suffered from local necrosis at the site of injection but no other lesion of importance.

An attempt to sensitize a rabbit by alternating small and large doses of T.N.T. failed.

The three T.N.T. isomers were separately injected into cats. The lethal dose of α-T.N.T. was greater than that of β or γ but repeated small doses of α more constantly produced drowsiness, loss of appetite, and loss of weight. Both β- and γ-T.N.T. produced a yellow pigmentation of the blood serum and subcutaneous tissues.

Local necrosis at the sites of injection were set up by all the isomers dissolved in toluol.

The two major lesions of T.N.T. poisoning were approximately produced in two experimental animals only.

A cat given γ-T.N.T. acquired shortly before death the exact and typical blood changes of aplastic anaemia. The rapid alteration in the circulating blood of this animal was most striking and simulated precisely the human cases.

In one cat only were any striking changes induced in the liver, a marked necrosis and fatty degeneration of the central lobules being found post-mortem. This animal received α-T.N.T. together with subcutaneous injections of dead colon bacilli.

VI. EXPERIMENTAL WORK UPON T.N.T. POISONING AND THE PATHOLOGICAL CHANGES INDUCED BY T.N.T. IN HUMAN BEINGS.

By P. N. Panton, M.B., B.C., Cantab.

CONTENTS

	PAGE
Experimental	72
The Mode of Absorption	72
The Exact Nature of the Toxic Substance	73
Pathological	79
References	88

Experimental.

The problems which in the course of this investigation particularly called for experimental work were in the first place the manner in which the poisonous substance was absorbed—whether by inhalation, ingestion, or through the skin, then the exact nature of the poison and finally the mode by which the toxic effects were produced.

The Mode of Absorption.

The three paths to be considered were inhalation, ingestion, and absorption through the skin, and to any one who has visited a T.N.T. factory the last path is the most obvious. That nitro-bodies such as dinitrobenzene can be absorbed through the skin of cats and human beings had been previously and conclusively proved by White and Hay (1). The proof that T.N.T. is mainly absorbed in this manner was adduced by Dr. Benjamin Moore (2) and his repeated advocation of skin protection, coupled with more cleanly —and incidentally safer—methods of handling T.N.T. had the greatest influence in restricting the dangerous results of T.N.T. absorption. The experiments on T.N.T. absorption made by Dr. Moore and his co-workers were both animal and human, the latter being entirely convincing both that T.N.T. can be absorbed through the unbroken skin in ordinary manipulation, and that poisonous results can arise from such absorption. The test of absorption was furnished by the appearance of Webster's reaction in the urine and the toxic sequel was manifested in the person of Dr. Moore himself (2).

The amount of T.N.T. that can be ingested by the introduction of dirty fingers into the mouth is evidently small compared to that

constantly rubbed through large exposed areas of skin and the amount of dust that could be swallowed by an operative during a day's work was shown by Dr. Moore, in dust extraction analysis, not to exceed 10 mg.

Similarly the amount possible to be inhaled by those working near molten T.N.T. was shown to be very small, though some of the worst early cases I saw were among this class of operative.

Koelsch (3), while admitting skin absorption, laid stress upon the importance of inhalation, but he was convinced of the non-toxic properties of T.N.T. and later (4) obsessed by the toxicity of tetranitromethane which occurs as an impurity.

The problem on the whole seems fairly comparable to the older controversies on lead poisoning. The painter absorbs lead constantly and considerably through the skin, he occasionally adds small quantities of it to the food he takes with his meals, but he may inhale it to a dangerous extent in the process of burning off the paint. Similarly the main path of absorption of T.N.T. is undoubtedly the skin, further small amounts may be accidentally ingested and to a more problematical extent the vapourized substance may be inhaled. It must be admitted, however, that the minimal fatal dose for a human being may be minute. There are very definite instances of major poisoning in which the amount absorbed through the skin must have been infinitesimal and the poisoning presumably resulted from the amount ingested. In view of the minute bulk of T.N.T. which may be fatal to a susceptible person, all channels of absorption are important, even though the main channel for the majority is the skin.

The Exact Nature of the Toxic Substance.

Here again the most obvious, though not necessary correct, solution is that the actual poisonous substance is T.N.T., since T.N.T. forms the great bulk of the explosive powder even in its commercial state. T.N.T. had, however, primarily been considered to be non-toxic in marked contrast to dinitrobenzene, and certain other impurities and decomposition products of T.N.T. had to be considered.

A possible source of poisoning to which attention was called in an early stage of the inquiry was the inhalation of fumes given off by T.N.T. Nitrous fumes in amount detectable by the sense of smell are given off by T.N.T., and the toxic effect of T.N.T. was ascribed to these fumes by, among others, the managing director and staff of the National Shell Filling Factory at Chilwell. As has been pointed out by Dr. Moore (10, p. 70) workers in processes involving the inhalation of much larger quantities of nitrous fumes than ever obtained in T.N.T. filling are not affected by either jaundice or anaemia, and the nitrous fumes extracted by him from the neighbourhood of molten T.N.T. were infinitesimal. Rabbits and white rats were exposed by me to a percentage of nitrous fumes in a fume chamber greater by far than ever could occur in any T.N.T. process, and though it was found that the animals, even with small quantities of gas, showed cyanosis and gave a positive Haldane test for methaemoglobinaemia, neither jaundice nor anaemia was pro-

duced in any instance. The exposures of these animals to NO fumes extended over 3 months, each exposure lasting about 3 hours. In spite of the expressed opinion of the staff at Chilwell, the danger of nitrous fumes in T.N.T. filling processes can, I think, be disregarded as a cause of major poisoning.

Another T.N.T. impurity to receive attention, both in this country and in Germany was tetranitromethane. Koelsch (3) considers the severe toxic effects to be due to impurities, of which he mentions nitrated benzols and non-nitrated hydrocarbons, remains of nitrous fumes, and tetranitromethane. The action of $C(NO_2)_4$ is, however, a direct one on the respiratory passages, and Koelsch failed to obtain any evidence of its action except as a possible cause of some of the minor forms of T.N.T. poisoning, including cyanosis. There is no evidence or suggestion that either toxic jaundice or aplastic anaemia can be produced by this substance, and the amount of tetranitromethane obtained in samples of T.N.T. in this country was small. Similarly the benzols occur in very small amount only. The only substance used in bulk with T.N.T. is the ammonium nitrate which is mixed with T.N.T. in amounts up to 80 per cent., but this substance is commonly held to be quite innocuous. A rabbit given by me $\frac{1}{2}$-grm. doses daily (with certain intervals) under the skin for 3 months was quite unaffected.

By a process of elimination the conclusion is reached that the poisonous substance is T.N.T., but certain difficulties obstruct the direct proof. The main difficulty in the first place was the original failure to reproduce the major lesions of T.N.T. poisoning in animals, and this has only been partially surmounted. Further it has been difficult to explain the rarity of the lesions among the large numbers handling the powder. One would like also to account for the strange latency, often enduring months, before symptoms arise. Before describing the nature of the animal experiments performed the possible explanations to which they were directed may be mentioned.

The metabolism of the drug within the body may be different in different species of animals. It has long been known that toluylene-diamine affected dogs quite differently to guinea-pigs.

Explanation was required for the low rate of incidence and the fact that the workers affected became so within a few weeks of commencing work with the powder, severe poisoning among those who had been at work for many months being practically unknown. The usually accepted explanation is that of individual susceptibility, though the phrase can hardly stand for an explanation, and the obvious analogy is the prevalence of alcohol drinking and the comparative rarity of alcoholic cirrhosis of the liver. Various possibilities suggested themselves, and among them that poisoning might arise by a species of sensitization by which operatives taking small doses of poison at intervals might render themselves sensitive to a subsequent dose ; such an explanation would be most unfavourable to the generally adopted system of alternation of labour, and in fact alternation did not increase poisoning in factories. Another suggestion was that the susceptibility of the individual depended upon a second factor, such a factor being a possible infection of the biliary passages by a coliform bacillus rendering the liver more

susceptible to the action of the T.N.T. Further factors were differences in the permeability of the skin of individuals and individual differences in the metabolic processes by which the drug was dealt with. Another possibility was that of variation in the composition of the drug itself, and attention was here directed to the separate toxic effects of the three T.N.T. isomers. The results of the various experiments made will be briefly recorded.

That the metabolism of the drug varies in different species of animals was proved by Mr. Webster and Dr. Wyon, working under the auspices of Dr. Dale and Dr. Barger. The animals used in the early experiments were rabbits, guinea-pigs, and rats, and it was subsequently shown that cats are very much more sensitive to T.N.T. than rabbits. It was found that rabbits taking T.N.T. excreted in the urine a chromogen giving a reaction identical with that found in man, but that this substance is not present in the urine of the more susceptible cat. The difference in susceptibility of the two species of animals helped to explain the difficulty in reproducing the lesions of T.N.T. poisoning in the rabbit. Since man stands in an intermediate position between herbivors and carnivors it is conceivable that individual differences in the diet and metabolism of man might produce different susceptibilities to the drug.

Dr. Moore found Webster's test to be positive in the urines of all those absorbing T.N.T. but to a different degree in different individuals. He further found different degrees of permeability of the skin, and it is possible that some cases of poisoning arose by an overdose owing to an abnormal permeability of the skin of the individual.

Attempts by Dr. Vaidya and myself to sensitize rabbits by alternating small and large doses of T.N.T. (from 100 to 750 mg. given hypodermically) at intervals of a week to ten days failed to have any other effect than the more regular mode of administration.

The suggestion of Dr. Laidlaw that the introduction of colon bacilli in addition to the T.N.T. might succeed in producing the liver changes was tried by Dr. Bates and myself. Two cats were injected over periods of six months with T.N.T. α, and at the same time were given large doses of *B. coli*. In one cat some of the bacterial injections were made intravenously with live bacilli, and in the other cat hypodermic injections of dead organisms were given. Changes were produced in the liver of one of these cats and are referred to later.

The different effects of the 3 T.N.T. isomers were investigated by Dr. Bates and myself with the advice and assistance of Dr. Dale. The great bulk of the T.N.T. powder used consisted of the symmetrical T.N.T. α. The β and γ-T.N.T.s vary in amount from a mere trace to 4 per cent. in an exceptional sample analysed by Mr. Tutin. If it could have been proved that the β and γ substances produced in cats lesions of toxic jaundice or of aplastic anaemia whereas the α-T.N.T. did not, the problem of the precise nature of T.N.T. toxicity would have been a little nearer to solution. The reproduction of the human lesions in animals has turned out to be extremely difficult. Dr. Moore, in a large number of experiments, mainly with rabbits, guinea-pigs, and rats failed to reproduce the liver and blood changes except in one possible instance

when certain liver changes were found.[1] Dr. Vaidya and myself similarly failed to reproduce the lesions in rabbits which appear particularly resistant to the drug. Cats were known to be more susceptible and it was found possible to produce some liver changes, and in one cat typical central necrosis of the liver lobules, though never the massive necrotic action of the human cases, and one typical example of aplastic anaemia was developed. The results of the experiments will be given very briefly, but it must be recognized that the number of animals experimented upon were wholely inadequate for conclusive results. The amount of work involved by even a small number of experiments was more than could be accomplished in the time at our disposal.

Thirteen cats were injected subcutaneously with T.N.T., and since the T.N.T. powder was dissolved in toluol, 1 cat was injected with toluol only as a control. It was particularly attempted to reproduce either aplastic anaemia or toxic jaundice or both.

The blood condition may be considered first. In 8 cats periodic blood examinations were made, and in 7 no specific blood changes were produced. The eighth case is of considerable interest. A cat had been given for from 9 to 10 weeks small doses of γ-T.N.T. by Dr. Dale. The blood was examined and the following result found:

Red cells	6,750,000 per c.mm.
White cells	22,800 per c.mm.

Differential count:

Polynuclear neutrophils	89·5%
Small lymphocytes	5·0
Large lymphocytes	0·5
Large hyaline	5·0

One normoblast was seen among 200 leucocytes and some polychromatohhilia.

A comparatively normal blood count for a cat.

Six days later the cat was very ill, and the following striking change in its blood condition was found to have occurred:

Red cells	1,937,500 per c.mm.
White cells	600 per c.mm.

Differential count:

Polynuclear neutrophils	8%
Small lymphocytes	58
Large lymphocytes	18
Large hyaline	16

Sixteen normoblasts were seen in 100 leucocytes. Occasional cells showed polychromatophilia.

The cat was killed soon after this blood count was done and the shaft of the femur was found well filled with red marrow. Films showed almost entirely mononuclear cells of lymphoid and endothelial types, scanty eosinophiles mainly mononuclear, numerous normoblasts and occasional megaloblasts.

The changes in the blood as shown by the rapid loss of red cells without marked change in their morphology, and the extreme

[1] These liver changes occurred in a rabbit and were identical with those produced in one of our cats. I am unable, however, to find any record of the experiment from which the liver specimen was obtained.

leucopenia, together with the almost total disappearance of the granular marrow cells, are identical with the changes in the human cases.

On the other hand a cat injected with γ-T.N.T. by Dr. Bates and myself, in which the blood was periodically examined over a period of 4 to 5 months, showed no blood changes whatever during life. These two cats were the only animals treated with γ-T.N.T. in which the blood was examined.

No blood changes were found in the cats treated with T.N.T. *a* or T.N.T. *a* and *B. coli*, T.N.T. β and toluol.

The liver changes. The histology of the tissues examined was in the main referred to Dr. H. M. Turnbull, and is reported at greater length by Dr. Bates and myself elsewhere. In the rabbits and rats examined no liver changes of note were produced. In six cats and three kittens injected with T.N.T *a*, β, and γ, and in a control cat injected with toluol no hepatic lesions were produced which could be certainly ascribed to the drugs given, and in only one case was any myeloid reaction found in the tissues.

Two other cats were given T.N.T. *a* and *B. coli* in addition, and in one of these definite liver changes were produced. The changes consisted in a fatty degeneration and necrosis of the central zones of the hepatic lobules, haemosiderosis of the liver and a myeloblastic activity of the marrow and accessory blood-forming organs. The hepatic changes were considered to be due to the toxic agents employed and to be independent of such anaemia as might have resulted from the disturbances of blood formation. The second cat showed myeloblastic activity but no liver changes of importance. The hepatic lesions in the former case were very similar to those produced in a rabbit by Dr. Moore and the results of these few experiments in which bacterial suspensions were added to the T.N.T. injections, do not in reality lend much weight to the views that a second factor is necessary to produce the lesions, and that the factor is a bacterial toxin.

The effect upon the cats of these drugs, other than the histological changes in the blood and viscera, may be briefly mentioned. Loss of weight was a constant feature and was well marked. One cat, treated with β-T.N.T. over 3 months, during which time it received in all 3,125 grm., went from 5,340 grm. to 1,930 grm. On the other hand the control cat injected with toluol lost weight appreciably.

Cyanosis, as evidenced by duskiness of the gums, was noticeable in the early stages of chronic poisoning, but later the cats became apparently jaundiced, and the cyanosis less obvious.

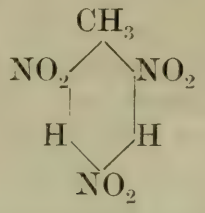
Jaundice, as evidenced by a yellow colour of the mouth and of the blood serum, was constantly present but in varying degree. The serum of a kitten, injected by Dr. Dale, was of a bright yellow colour, and the serum of our animals when examined was definitely yellow when compared to the white serum of a normal cat or of the cat receiving toluol. The skin and subcutaneous tissues post-mortem were sometimes noted to have been deeply yellow. The yellow colour, however, was not due to the presence of bile pigments in the blood but to a combination of the T.N.T. with the body proteins producing a bright yellow reaction. The yellow colour

of the blood serum was particularly marked in all the β and γ T.N.T. cases and was not noted in any of the cats treated with pure α T.N.T. Local necroses at the site of inoculation of the drugs were extremely common with all the isomers. These necroses were usually aseptic but occasional considerable abscesses developed, and the animal had to be killed.

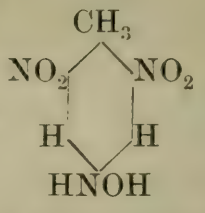
The effect upon the general condition of the animals was, in the case of small repeated doses, e. g. from 50 mg. upwards, to produce torpidity and listlessness, and unless frequent intermissions in the treatment were made the cats became drowsy, went off their food, lost weight rapidly and died. The necessity for intermissions was particularly noticed in the cats treated with T.N.T. *a*. Although the lethal dose for the cat of T.N.T. β or γ is smaller than that of α, repeated small doses are worse tolerated in the case of *a*.

The metabolism of T.N.T. within the bodies of man and animals has been already referred to. The following brief account is taken from the work of Webster and Wyon, investigating under the supervision of Drs. Dale and Barger, and the conclusions given in their report have been revised from subsequent information given me by Dr. Dale.

After absorption through the skin T.N.T. is possibly reduced to dinitrohydroxylaminotoluene.



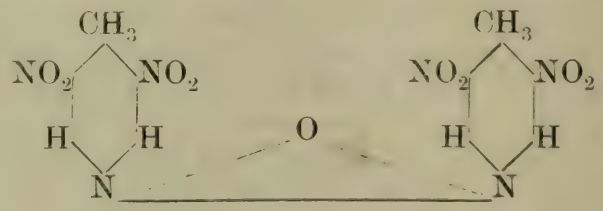
a Trinitrotoluene.



Dinitrohydroxylaminotoluene.

The latter substance has not, however, been identified in the urine, but appears as an azoxy compound which can be formed by condensation of dinitrohydroxylaminotoluene by an acid or alkali.

The azoxy compound has the formula



and is excreted in the urine in conjugation with glycuronic acid.

The azoxy compound is the substance which gives Webster's test. In animals, however, who are given T.N.T. in massive doses, only a small portion of the T.N.T. excreted appears in this form, the bulk of the excreted derivative has a different composition, the nature of which is discussed elsewhere in this report, and does not give Webster's test.

The azoxy compound has been isolated from the urines of T.N.T. workers, of rabbits, and of a monkey. It is absent from the urines of cats.

In the free condition the azoxy compound has about 3 times the toxicity of the original T.N.T.

The lethal dose of T.N.T. for a cat is about 0·25 grm. per kilogram body weight, whether given hypodermically or by the mouth.

The different modes of metabolism of T.N.T. in man, the rabbit, and the cat, are worth considering further in conjunction with our clinical knowledge of the effects of T.N.T. on man.

Webster's test for the T.N.T. derivative in the urine was originally considered by Dr. Moore to be a test for the susceptibility of the individual. Those operatives giving an intense urinary reaction would have been, on this assumption, refused T.N.T. work. Subsequent investigation renders the opposite of this hypothesis more probably correct. The great majority of human beings metabolize T.N.T. in a similar mode to the rabbit. Both man and rabbit are comparatively immune to the poison. Is it not possible that the very exceptional man metabolizes T.N.T. in an exceptional manner? Unfortunately we have no records of operatives who failed to give the T.N.T. reaction in the urine and who subsequently proved susceptible, but there is some clinical evidence in support of the theory.

Dr. Moore found a positive Webster's test in the urine up to two weeks after contact with T.N.T., yet in not a single case of toxic jaundice has the test been found positive in the urine, and numerous cases have been examined in this respect within two days of leaving work. Further, in one case of severe toxic jaundice, which subsequently recovered, T.N.T. was rubbed into the skin, and Webster's test continued to be negative in the urine.

The evidence is certainly not complete and is open to criticism. Dr. Castellain has informed me of 14 operatives in liquid T.N.T. whose arms were covered in the T.N.T. solutions, none of whom gave Webster's test and all of whom remained healthy, but poisoning from this particular branch of T.N.T. work appears to be rare, and it is conceivable that T.N.T. dissolved in paraffin is less readily absorbed by the skin. Dr. Castellain further suggested the possibility that bile in the urine might interfere with Webster's test, and there is the further possibility that the disorganization of the liver interferes with the normal metabolism.

On the whole the theory of abnormal individual metabolism seems to have more bearing upon the explanation of individual susceptibility than any question of abnormal variation in the powder either from the presence of impurities or the particular action of isomers.

Pathological.

The path by which T.N.T. is absorbed, the more concise account of its mode of metabolism in the bodies of man and animals, and the exact nature of the poison which produces the visceral disturbances to be described are more conveniently discussed in the experimental section. It may be briefly stated here that the main channel of absorption is the skin, and that the actual poisonous substance is almost certainly T.N.T. itself.[1]

[1] T.N.T. is here meant to include the symmetrical T.N.T. which forms the great bulk of the powder and the isomers β and γ T.N.T. which, in an exceptional sample examined by Dr. Dale, constituted as much as 4 per cent. of the powder.

The pathological effects of T.N.T. absorption by the human subject are evidenced locally : in the process of elimination of the substance, and by its action on certain internal organs, particularly the liver and bone-marrow.

The local action of T.N.T. produces universally a yellow discoloration of the parts of the body exposed to the powder. In susceptible individuals a localized dermatitis is set up in addition to the discoloration. Further Dr. W. J. O'Donovan has informed me of three fatal cases of Ludwig's angina occurring among T.N.T. workers, in all of whom there was severe oral sepsis, and it is possible that the local action of T.N.T. powder upon the mouth affected the original course of the disease. The local necrosing action of T.N.T. is frequently seen in experiments on animals and is referred to later.

In the process of elimination certain symptoms and physical signs dealt with in the clinical section of the report may be set up. Of these, gastritis may be due in some cases to the irritant action of the T.N.T. directly absorbed from the mouth into the stomach, but probably the more usual path to the stomach is by way of the skin. The drug is partly excreted by the skin, and a red staining of the clothes in the axillary region is not uncommon, the red colour following the addition of alkali when the clothes are washed. Rabbits taking T.N.T. excrete large amounts of a bright pink pigment which collects upon and adheres to the fur. The faeces of operatives in T.N.T. factories do not appear to have been systematically searched for T.N.T., but the unchanged substance has been detected by Dr. Moore (2) in the faeces of rabbits which had received large doses by the mouth. The unchanged substance cannot be detected in the faeces or viscera of persons recently employed on T.N.T.

The main channel of elimination is the urine. T.N.T. is not excreted as such in the urine since extraction by ether fails to remove it. The trinitrotoluene after preliminary reduction of one of the nitro groups is excreted in the urine in conjugation with glycuronic acid. The reduced T.N.T. can be liberated from the glycuronic acid by treatment with an acid, and a colour reaction has been devised for its detection. This test for the detection of T.N.T. as it is eliminated in the urine was worked out by Mr. T. A. Webster (5), and is performed as follows :

Measure out 12½ c.cm. of the urine in a measuring cylinder, then add 12½ c.cm. of diluted sulphuric acid, made up by mixing 20 c.cm. of strong sulphuric acid with 80 c.cm. of water. Pour the mixture of urine and acid into a separating funnel of 100 to 150 c.cm. capacity and provided with a stopcock ; add to the mixture 10 c.cm. of ethylic ether (the ordinary ether made from methylated spirit is sufficiently pure for the purpose), shake up well and allow to settle ; take out the cork or stopper from the top of the separating funnel, open the stopcock at the bottom and allow the mixture of acid and urine to run off, then turn the stopcock off so as to retain the ethereal solution in the separating funnel. Now add 25 c.cm. of tap water to the ethereal solution in the separating funnel and shake up again to remove the traces of the mixture of urine and acid, and allow to settle again for two or three minutes, then run off the water by opening the stopcock, retaining the ether in the funnel. Finally, let the ethereal solution flow into an ordinary test-tube and try for the presence of T.N.T. in it as follows :

Prepare a solution of alcoholic potash by dissolving 4 to 5 grams of caustic potash in 100 c.cm. of methylated spirit or absolute alcohol. Where many tests are to be carried out this solution may be made by having a stock saturated

solution of caustic potash, and adding, when a fresh quantity of the reagent is required, 10 c.cm. of this to 90 c.cm. of alcohol.

To the ethereal solution obtained as above described 5 c.cm. of this alcoholic solution of potash are added. When T.N.T. is present a purple coloration is at once developed, varying in intensity according to the amount of T.N.T. present, from the faintest trace to a deep purple. The colour changes rapidly from the purple to a brown colour, and it has been found that the best results as to intensity are obtained by judging rapidly after the colour is struck.

Webster's test was subsequently shown by Frank Tutin (6) to have a source of error since various purgatives, including rhubarb, senna, and cascara contain anthraquinone derivatives which give a colour test very similar to that produced by T.N.T. Tutin devised the following modification which excludes anthraquinone derivatives :

The urine should first be extracted with two successive portions of ether. This treatment removes any T.N.T. which may be present from accidental contamination, and also chrysophanol. The urine is then acidified with hydrochloric or sulphuric acid and again extracted with ether. During these extractions the addition of alcohol is usually necessary to cause the separation of emulsions. The ethereal extract of the acidified urine must then be extracted twice with dilute aqueous sodium carbonate, washed with water, and then treated with alcoholic potash, when the characteristic violet colour will be produced if derivatives of T.N.T. were present in the urine. If anthraquinone derivatives were present the sodium carbonate extracts will have a reddish-violet colour.

The toxic actions of the major kind by T.N.T. upon the internal organs are rare. The actual incidence is dealt with elsewhere, but it may be mentioned here that with exceptional opportunities, and in the space of about one year, I saw 30 examples only. The rarity of these serious effects led to the pre-war belief that T.N.T. was devoid of serious poisonous properties. The explanation of the rarity will be discussed later. The main toxic action of T.N.T., other than the production of minor ailments such as gastritis and dermatitis, is chiefly manifested upon the liver and the blood-forming organs.

The changes produced in the blood and blood-forming organs may be summarized from papers by Dr. Matthew Stewart (7 and 10) and myself (9 and 8).

In 50 women at work and free from symptoms no changes in the red cells nor in the haemoglobin percentage were found. The only change found in the blood was a moderate leucocytosis, giving an average of 10,000 leucocytes per c.mm., the highest total counts showing a relative increase in the polynuclear neutrophils. The deductions drawn were that a daily absorption of T.N.T. by the majority of individuals has no destructive action upon the blood, and has a stimulating action upon the granular leucocytic tissue of the bone-marrow.

Cyanosis was found to be common. No fewer than 78 out of 100 women examined on a cold day showed some degree of cyanosis, and of these 18 were strikingly cyanosed. The cyanosis follows the absorption of T.N.T. and is due to a mixture of NO haemoglobin and methaemoglobin in the circulating blood. Marked cyanosis may be present without symptoms, and a serious degree of methaemoglobinaemia does not seem to occur. Koelsch (3) failed to produce methaemoglobinaemia in animals, and concluded that cyanosis and other

symptoms of poisoning did not occur with pure T.N.T., but were due to impurities in the war product. These views will be again referred to later. Dr. Moore (2), one of the first to call attention to the frequency of cyanosis in T.N.T. workers, assumes that the methaemoglobin-carrying red cells are broken up, and that a compensatory increased production of new cells puts a strain upon the red marrow. He follows out his assumption by ascribing aplastic anaemia to exhaustion of the red marrow following this blood destruction and toxic jaundice to liver exhaustion following upon the excessive haemoglobin metabolism. Dr. Moore's deductions are accompanied in his paper by arguments which appear to me fallacious, and his original assumption of constant blood-destruction is unsupported by any proof and directly opposed to available evidence. Large quantities of blood withdrawn at regulated intervals may subsequently lead to an erythraemia, though I have not found this to occur among ' donors ' used for repeated transfusions of blood, but continuous abnormal blood destruction in human pathology, including destruction consequent upon malarial infection, invariably leads to anaemia, and these cyanosed workers are neither anaemic nor polycythaemic. Also T.N.T. anaemia, when it does occur, is of a rapidly fatal kind, accounted for by destruction of the bone marrow rather than the blood. Methaemoglobinaemia may be pushed to a marked degree without producing any apparent red cell destruction. From the experiments of Haldane, Makgill, and Mavrogordato (21) it is quite clear that mice rendered methaemoglobinaemic to an extreme degree (by hypodermic injection of sodium nitrite 74 per cent. of the haemoglobin was converted into methaemoglobin) and subsequently recovered by exposure to oxygen, must have exhibited the property of the red cell to recover from methaemoglobin combination without permanent injury. The freedom of aniline workers from toxic jaundice is also noteworthy.

Cholaemia. Twenty per cent. of workers showed slightly tinged sera, a very frequent phenomenon among normal people ; 3 per cent. only showed sera at all deeply tinged with bile, and the conclusions drawn were that a bile-tinged serum was insufficient evidence of T.N.T. poisoning, and that no proof was obtained of any constant minor action of T.N.T. upon the liver of the average worker.

Toxic jaundice. In 24 cases of toxic jaundice, as well as in certain other examples of minor T.N.T. sickness, the blood was entirely normal, except for the presence of bile pigment in the sera. There was no increased fragility of the red cells. In one severe and seven convalescent cases the Wassermann Reaction was negative. Dr. Jacob, in Nottingham, found the Wassermann Reaction positive in three consecutive cases, and the probability was that these three cases were syphilitic also. In the cases that recovered, bile pigment was commonly found to persist in the serum for many months. The conclusion arrived at was that the jaundice was not haemolytic in origin but was a toxic jaundice consequent upon the liver destruction.

Aplastic anaemia is the rarest and most fatal of the T.N.T. complications ; it is doubtful if recovery ever occurs. It is characterized during life by a primary anaemia with a high colour index, rapidly progressing to an extreme degree. The red cells are unaltered

morphologically, the granular white cells are diminished until a total leucocytic count of 1,000 per c.mm. may be reached, of which the polynuclear neutrophils form only 4 per cent. The serum is colourless, often in marked contrast to the greenish-yellow serum present in pernicious anaemia. Post-mortem the blood-forming marrow is reduced to an extreme degree.

It would appear that haemolysis occurs, at any rate in the terminal stages, because in the cases in which the aplastic nature of the anaemia was proved, there was a conspicuous haemosiderosis of the liver. In one case, however, in which the anaemia was in all probability aplastic, no haemosiderosis of the liver could be detected. Further, in the proven cases of aplastic anaemia pigment derived from haemoglobin was not demonstrable in the blood during life. The essential lesion is a reduction of the blood-forming marrow. There can be little doubt that this is due to the direct action of the poison upon the blood-forming cells in the marrow. Direct evidence of injury to the marrow cells is afforded by the extensive phagocytosis of nucleated cells found in one of the two cases in which a systematic microscopic examination of the bone-marrow was made. The aplastic anaemia may occur quite independently of the jaundice, though persons susceptible to the toxic action upon the liver are more susceptible to the action upon the marrow also. Thus of 42 cases of toxic jaundice observed by Captain Stewart and myself 7 or 16·6 per cent. developed aplastic anaemia subsequently. The anaemia may, however, arise in the absence of any clinical or histological liver changes. The well-known latent period which may elapse between exposure to T.N.T. and the appearance of jaundice is usually exceeded by the interval before the appearance of anaemia. Consequently in some jaundiced cases it has been possible to watch the subsequent development of the anaemia from the time of the first appearance of any blood changes. Purpura has been a prominent clinical feature of some of the aplastic anaemia cases, and is probably of the same nature as may occur in other severe forms of anaemia, and particularly in the acute leukaemias.

The post-mortem changes. The changes found in the fatal jaundiced cases, together with the post-mortem findings in aplastic anaemia, may be considered together. The histology of these conditions has been worked out by Dr. H. M. Turnbull, and the following description is based upon a paper read by him before the Royal Society of Medicine (10) with certain modifications and additions subsequently communicated to me by him. At the same meeting a communication was also made by Dr. Stewart whose paper is accompanied by illustrative plates. Reference may also be made to the report of a case in the United States by Martland (11), giving a brief histological account of the liver changes in a toxic jaundice case, and to a Canadian case described by Dr. Horst Oertel (12), who concluded that T.N.T. acted, not upon the parenchyma of the liver, but primarily upon the endothelium of the blood-vessels and lymphatics. Dr. Oertel's case does not appear to be a pure case of toxic jaundice, and though no mention of any blood examination is given, the account of the case suggests toxic jaundice, with partial recovery and subsequent death from aplastic anaemia.

The changes described by Dr. Turnbull in 7 cases of severe jaundice are given first.

The Liver was greatly reduced in size. Externally were large areas of depression over which the capsule was slightly thickened and opaque, and projecting areas over which the capsule was transparent and revealed a lobular pattern. The cut surface showed similar raised and depressed areas, some of the former appearing as small projecting nodules. The depressed areas showed grey points, corresponding to portal sheaths, set close together in a firm, red ground. The raised areas were soft, and showed a lobular pattern marked out by dark yellow or bright red centres and bright yellow peripheries; elongation and anastomosis of portal sheaths were often recognizable. The largest area showing a lobular pattern lay, except in one case, in the anterior aspect of the upper part of the right lobe. A great portion, from one-third to two-thirds, of the right lobes was destroyed. The whole left lobes were greatly shrunken and consisted almost entirely of the depressed tissue. The spigelian lobes, with one exception, were completely or almost completely destroyed. The weights of the livers varied from 17 oz. (in a male aged 14) to 31 oz. (in a male aged 35). Microscopically there was degeneration and necrosis of parenchyma associated with infiltration and fibrosis. Destruction of parenchyma was complete in the sunken and incomplete in the raised areas. The process commenced with a degeneration partly fatty, partly dropsical, and partly hyaline, which began round the central and other hepatic veins, and might extend from lobule to lobule at the points farthest from the portal systems. The more severe and acute the degenerations and subsequent necroses were, the less appeared to be the part played by fatty degeneration. In the degenerate areas the capillaries became dilated and small haemorrhages were frequent. A cellular infiltration with polynuclear leucocytes took place, and later a fibrosis accompanied by the cells of chronic inflammation. The final fibrosis in these cases in which the hepatic destruction proved fatal, was delicate. In the depressed areas the hepatic cells in the peripheral zones of the lobules were destroyed as well as those in the centre, and relatively little fat was present. No free iron pigment was demonstrated in any liver. Regeneration in the raised areas was slight or absent, and most evident where the duration of the lesions was longest, as estimated by the density of the fibrosis.

The bile canaliculi were distended by bile, and more obviously in the hepatic columns next the central necrosis than in those near the portal systems. Bile was rarely present in the portal ductules, almost all of which were collapsed. The portal ducts were empty of bile; in one case there was catarrhal desquammation, in another slight infiltration with leucocytes. It was concluded that while the escape of bile into the blood as the result of parenchymatous disintegration could not be excluded, the histological evidence was in favour of the assumption that the jaundice was obstructive. There was no evidence that the jaundice was of haemolytic origin.

The gall bladder in the two cases in which it was examined microscopically showed in one mucous catarrh, and in the other subacute inflammation. The gall bladder was available for micro-

scopical examination in a third, subsequent, case; subacute inflammation was present.

The pancreas. Dr. Turnbull has examined the pancreas microscopically in six cases of acute obstruction of the liver by trinitrotoluene. Digestion was present in all. In three cases it was universal, being in two associated with infiltration by putrefactive bacteria. In the three other cases there were numerous focal areas of digestion. The tissues were in no case perfectly fresh when fixed, and there was no histological evidence of inflammation within the pancreatic tissue, except mucous catarrh of the duct of Wirsung in one case. The pancreatic auto-digestion may, therefore, have been a post-mortem phenomenon. Similar pancreatic digestion was found, however, in cases of acute destruction of the liver attributed to salvarsan and other poisons, and in some of these cases it undoubtedly occurred before death. It is possible, therefore, that the pancreatic digestion affords additional evidence of inflammation of the gall-bladder and common bile-duct in cases of destruction of the liver by trinitrotoluene and other poisons.

The kidneys, examined in six cases, were enlarged, rounded, and flabby. The breadth of the cortex was increased in relation to that of the medulla. Fatty degeneration was present in all, being greatest in the first and second convoluted tubules and the ascending loops of Henle, less in the glomerular endothelium and in the collecting and discharging tubules.

The myocardium, when examined, showed extensive fatty degeneration.

Haemorrhages, petechial and numerous, were present in three cases, and no records were available in the remaining four cases. The cause of the haemorrhages was probably a fatty degeneration of capillary endothelium. Such a degeneration was found in the cerebral capillaries in two cases in which the *brain* was available for microscopic examination.

In the same paper is a record of the examination of three cases of severe anaemia without jaundice. In two cases a complete necropsy was made by Dr. Turnbull, and the anaemia was proved to be aplastic. A summary is given of the microscopic findings in the first of these two cases (male, aged 32). The visceral changes were those of a severe anaemia, fatty degeneration of vascular endothelium being very conspicuous. There was very little haematogenous marrow. In such as was present erythroblastic activity preponderated, and megakaryocytes were very scarce. In the marrow there were numerous plasma cells and large phagocytes. The latter contained pyknotic nuclei, erythroblasts, erythrocytes, and large granules of iron from ingested erythrocytes. Evidence of phagocytosis of erythrocytes was found also in the spleen and in the lymphatic glands, being very slight in the latter. The liver weighed 3 lb. 0¾ oz. The hepatic cells in the peripheral zones of the lobules were loaded with fine granules of iron. Dr. Turnbull considered that the destructive agent had acted upon the cells in the blood-forming organs, and also upon the circulatory erythrocytes. In the second case (male, aged 37) I had examined the blood during life and had diagnosed aplastic anaemia. The liver weighed 3 lb. 4 oz. The microscopic

examination of the tissues had not been made when Dr. Turnbull wrote his paper, but has been made since. The findings resembled closely those in the first case. Phagocytosis of red corpuscles in the lymphatic glands was greater, and in the spleen there were numerous cells containing coarse granules of iron. In the marrow there was abundant evidence of phagocytosis of erythrocytes, but in sections from the humerus, three ribs, the sternum, and two lumbar vertebrae, only six phagocytic cells were found to contain nucleated cells. There were fewer plasma cells in the marrow. The hepatic cells contained abundant granules of iron. In the kidney there was slight, subacute, parenchymatous nephritis.

In the third case (female, aged 38) no complete examination had been made of the blood during life, but the morphological characters of the blood were said to resemble those in the above two cases of aplastic anaemia. The marrow was not examined at the post-mortem. The liver weighed 2 lb. 5 oz. Portions of liver and kidney were received for examination. The liver contained areas of destruction similar to those in the cases of fatal jaundice, except that the fibrosis was much denser, and elastic fibrils had been formed therein. It also showed a fatty degeneration such as occurs in severe anaemia, but no iron was demonstrated by potassium ferrocyanide and warm hydrochloric acid.

Dr. Turnbull received, subsequently, tissue from three other cases of fatal anaemia in trinitrotoluene workers. In one case (male, aged 44) I had found the blood to show during life the typical changes of aplastic anaemia, and the nature of the anaemia was confirmed by examination of the bones by Dr. O'Donovan at the post-mortem examination. The liver (2 lb. 0½ oz.) contained extensive areas similar to those found in fatal cases of jaundice, but occupied by dense fibrosis ; elastic fibres had not yet been found in the fibrous tissue. Central fatty degeneration due to anaemia, and an abundant deposit of iron in the hepatic columns were also present. There was phagocytosis of red corpuscles in the submaxillary lymphatic glands, and a very little iron in the spleen. The marrow sent for examination was unfortunately taken from the lower end of the femur, which would normally have been free from haemotogenous elements at the age of the patient ; it was lipomatous and contained no phagocytes. In a second case (female, aged 21) the aplastic nature of the anaemia was proved by examination of the bone-marrow at the necropsy. The liver (1 lb. 11 oz.) was received for microscopic examination. It contained areas of extensive lobular destruction and of portal fibrosis similar to those in fatal cases of jaundice, but occupied by dense fibrous tissue in which elastic fibrils had been formed. It also contained abundant haemosiderin.

In the third case (female, age 20) no examination had been made of the blood during life, and the post-mortem examination was incomplete. There were numerous petechial haemorrhages on the trunk and limbs. There had been haemorrhage from the vagina for eight days before death. The anaemia was ascribed to haemorrhage following abortion. The liver weighed 2 lb. 3 oz. ; a portion was received for microscopic examination. This showed healed

small, trinitrotoluene lesions. It also showed a central fatty degeneration such as is found in severe anaemia, but no haemosiderosis. It is impossible to determine whether the anaemia in this case was due to trinitrotoluene, and whether it was aplastic. The severity of the anaemia and abundance of petechial haemorrhages favour aplastic anaemia rather than traumatic anaemia of eight days' duration.

Of six cases, therefore, of severe anaemia in subjects who had worked with trinitrotoluene, in four the anaemia was proved to be aplastic. In these four cases there was conspicuous haemosiderosis of the liver; in two there were also areas of previous hepatic destruction by trinitrotoluene. In both the two remaining cases there had been previous hepatic destruction, and haemosiderosis of the liver was absent. In one case the blood, examined during life, was said to resemble morphologically that of the aplastic anaemias, and the clinical course resembled closely that of the cases of aplastic anaemia. In the other the cause and nature of the anaemia are hypothetical.

The changes found in the livers of fatal jaundiced cases and the occurrence of jaundice followed by recovery has raised the important question of the permanent liver damage inflicted upon the recovered cases, and more important still the possibility that less severe but continually progressive changes are present in a considerable proportion of all T.N.T. workers.

As mentioned above, trinitrotoluene lesions were found in the livers of four of the six cases in which death was due to anaemia. In only two of these cases was the whole liver sent for examination; in both there was extensive scarring, suggesting to the naked eye the fibrosis of acquired syphilis. In one case (liver 2 lb. 0½ oz.) the patient had had an attack of toxic jaundice in April 1916, returned to the factory in August 1916 but did not handle trinitrotoluene, left the factory towards the end of December 1916, and died on February 16, 1917. In the other (liver 1 lb. 11 oz.) the patient had had an attack of toxic jaundice in September 1917, never returned to work, and died on March 26, 1918. In neither of the two cases in which portions only of the livers were received for examination was there a history of a previous attack of toxic jaundice; in one (liver 2 lb. 3 oz.) the patient had had an attack of T.N.T. gastritis on January 2, 1917, left work on March 31, 1917, and died on April 20, 1917. In all four cases the lesions were found on histological examination to have healed, and there was no evidence of progressive destruction of the liver. *Four livers of healthy T.N.T. workers instantaneously killed as the result of an explosion were examined by me and no abnormal changes were detected.* When one considers the small incidence of severe toxic jaundice and the somewhat larger and still small incidence of mild and recoverable toxic jaundice, it is possible that a number, somewhat larger still, of cases exist in which liver damage has occurred to a degree insufficient to cause symptoms. But such evidence as is available leads one to believe that any minor liver damage is recoverable and does not tend to progress.

A description of the pathological changes set up by absorption of

T.N.T. is scarcely complete without some mention of the similar action of other toxic substance.

The blood changes and liver necroses had been previously found to occur in benzol poisoning by Selling (13), Klemperer and Hirschfeld (14), and Heffter (15). The production of jaundice in animals by toluylenediamine was described by Stadelmann (16).

Blood changes, but of a different nature are commonly present among dinitrobenzene workers and were described by Malden (17). Fatal cases of toxic jaundice due to this drug are rare, but a case is described by Spilsbury (10), together with five fatal cases of 'aeroplane dope' poisoning with very similar liver changes. The poisonous substance in aeroplane dope was first shown by Heffter and Joachimoglu (18) to be tetrachlorethane. Marked destruction of the liver parenchyma has occurred, but to a very variable extent, in spirochaetosis ictero-haemorrhagica (19). Very similar lesions have been found in the liver in sporadic cases of acute yellow atrophy of unknown cause, and more recently in salvarsan poisoning. The lesions in the salvarsan cases are described by Dr. Turnbull (20) as almost identical with those set up by T.N.T. In phosphorus poisoning fatty degeneration is a more conspicuous feature.

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A Report on the Occurrence of Intestian Protozoa in the Inhabitants of Britain

UNIVERSITY OF TORONTO
FEB 23 1921



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15 Buckingham Street,
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A REPORT ON THE OCCURRENCE OF INTESTINAL PROTOZOA

IN THE

INHABITANTS OF BRITAIN

WITH SPECIAL REFERENCE TO

ENTAMOEBA HISTOLYTICA

BY

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CONTENTS

CHAPTER		PAGE
I.	INTRODUCTORY	4
II.	THE OLDER RECORDS OF THE OCCURRENCE OF INTESTINAL PROTOZOA IN THE POPULATION OF BRITAIN	17
III.	RECENT REPORTS ON THE OCCURRENCE OF INTESTINAL PROTOZOA IN THE POPULATION OF BRITAIN	21
	A. The Liverpool Reports	21
	B. The Later Reports	24
	(1) Birmingham	24
	(2) Brighton	27
	(3) Bristol	30
	(4) Leeds and Sheffield	31
	(5) Reading	37

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CHAPTER PAGE

IV. Summary of All Reports, and Discussion of Certain Points of Particular Interest 39
A. The Incidence of Protozoal Infections in Persons who have never left Britain 39
General Results 39
(1) *Entamoeba histolytica* 42
(2) *Entamoeba coli* 48
(3) *Endolimax nana* 49
(4) *Iodamoeba bütschlii* 50
(5) *Dientamoeba fragilis* 50
(6) *Giardia intestinalis* 51
(7) *Chilomastix mesnili* 52
(8) *Trichomonas hominis* 53
(9) Other Protozoa 54
B. The Spread of Infections in Britain 54

V. Recent Observations on the Occurrence of *E. histolytica* in France, Holland, and Germany 57

VI. Concluding Remarks 63

References 69

CHAPTER I. INTRODUCTORY.

Prior to the outbreak of war in 1914 little was known concerning the occurrence of intestinal protozoa in the population of the British Isles: for although many British investigators had devoted their attention to the study of the intestinal protozoa of man in tropical countries, it seems to have occurred to nobody, until very recently, to carry out similar investigations at home.

The reasons for this—which seems, at first sight, somewhat curious—are not difficult to discover. Diseases due to intestinal protozoa were not generally recognized as occurring in Britain. They were—and still are—regarded by most practitioners as peculiar to the tropics; and it therefore appeared unnecessary to look for the 'causes' of such diseases in a country wherein they seemed generally absent. Moreover, Protozoology, as a serious study, has usually been regarded as entering into the purview of those physicians only who devote themselves to the study of Tropical Medicine: and consequently there were few medical men who were able—or, if able, who saw any necessity—to study the intestinal protozoa of the resident population of Britain. And, on the other hand, those zoologists who might have been disposed to take up this work were usually debarred from doing so by the difficulty, or the impossibility, of obtaining adequate material.

All this was soon changed by the War. Large bodies of troops were raised in Britain and sent to all the theatres of military operations, where they contracted divers intestinal diseases with which later they returned home. On their return they were subjected to careful examination, in order to diagnose their

maladies with precision. Numerous workers were specially trained for this investigation, on the protozoological side, and in due course trustworthy figures were obtained for determining the incidence of infections with various intestinal protozoa among the particular class of case studied. The results have already been published in an earlier Report,[1] and elsewhere, and need not be further considered here. It will suffice to note that these returned soldiers were infected, to a considerable degree, not only with the common and well-known non-pathogenic intestinal protozoa, but also, and in a somewhat surprisingly high proportion, with *Entamoeba histolytica*—the 'dysentery amoeba'.

It was known before the War—chiefly through E. L. Walker's observations and experiments in the Philippines—that carriers of *E. histolytica* are not uncommon. Such persons, though harbouring active and facultatively pathogenic amoebae in the walls of their intestines, may present no clinical symptoms of infection: and they are, in consequence, indistinguishable from uninfected individuals unless their stools are subjected to careful microscopic examination. In 1914 it was still uncertain what percentage of persons who become infected with *E. histolytica* might be expected to develop into carriers of this type, and what proportion might be expected to suffer from dysentery or liver abscess, or to display other clinical signs of amoebiasis. Consequently, it was impossible to state, with any certainty, the precise significance of the high rate of infection with *E. histolytica* discovered in the convalescent dysenteric patients returning to Britain from the various theatres of war.

It soon became clear, therefore, that further facts, obtained from a different class of persons, were necessary for the proper comprehension of those already accumulated. The mere fact that some 25 per cent. of dysenteric convalescents, returned from Gallipoli or elsewhere, were infected with *E. histolytica* told us very little: for we were not told how many non-dysenteric cases from the same area were infected, nor what percentage—if any—of the whole body of troops was parasitized before setting out from England originally. In other words, we were presented with the end result of an experiment in which the earlier steps were unknown, and alongside of which no control experiments had been performed.

The first steps towards acquiring the necessary knowledge were taken early in 1916, when an attempt was made to examine the stools of soldiers invalided to England for disabilities other than dysentery. In those days it was difficult to examine non-dysenteric patients, as there were large numbers of dysenteric convalescents—whose diagnosis and treatment were urgent, on military grounds—awaiting examination in the hospitals and dépôts in this country. I succeeded,[2] however, in investigating some cases of the former class at No. 2 New Zealand General Hospital, Walton-on-Thames. The number was not great, but the results were somewhat surprising. I examined altogether

[1] See Dobell (1917). [2] See Dobell (1916).

110 non-dysenteric patients, and as a control a series of 90 dysenteric cases also—only 200 cases in all. The 'dysenteric' cases were all patients who had been invalided to England from Gallipoli and Egypt suffering, or having recently suffered, from 'dysentery'—diagnosed, in most cases, on clinical grounds only. The 'non-dysenteric' class consisted of patients invalided from the same areas for affections other than dysentery: but they included 40 cases of non-dysenteric intestinal ailments—the remaining 70 being wounded men and others with various non-intestinal complaints. The results obtained from all these different classes of patients were as follows:

Cases.	No. examined.	Infected with *E. histolytica*. per cent.
All cases	200	11·0
Dysenteric	90	10·0
Non-dysenteric	110	11·8
All intestinal ailments	130	8·2
All non-intestinal ailments	70	15·7

So far as these figures went, therefore, they did not warrant the conclusion that the cases being returned to England labelled 'dysentery' were more heavily infected with *E. histolytica* than those who were coming here by reason of their wounds or other non-dysenteric disabilities.

In the summer of 1916 I undertook, in collaboration with Dr. A. C. Stevenson, the examination of another series of non-dysenteric cases—the convalescent typhoid and paratyphoid cases at Hornchurch Dépôt. We examined the stools of 472 such patients, and found that 9·74 per cent. of them were infected with *E. histolytica*.

These observations were sufficient to indicate the importance of making further investigations along similar lines: and accordingly I urged all the protozoologists whom I had trained for the work of diagnosis at military hospitals to examine as many non-dysenteric cases as possible. Some of them did so, but owing to the requirements of the Army it was difficult to obtain adequate material for investigation. Mr. W. O. Redman King examined the stools of non-dysenteric patients in Graylingwell Military Hospital, Chichester.[1] He was able to study 59 men—mostly wounded—and found 8·5 per cent. infected with *E. histolytica*. Mr. R. E. Savage, at the 2nd Southern General Hospital, Bristol,[2] was also able to examine a number of paratyphoidal cases and a few patients suffering from wounds and other non-intestinal ailments, and obtained similar results. Miss M. W. Jepps, at Hampstead Military Hospital,[3] examined 65 soldiers invalided for 'irritable heart'; and among these she found—after an exhaustive inquiry—that no less than 24 were infected with *E. histolytica*. Some of these at least had never suffered from dysentery or other intestinal ailment. A few other workers made similar observations, but on a smaller scale. The most

[1] See Dobell (1917), p. 59. [2] See Hall, Adam, and Savage (1916).
[3] See Jepps and Meakins (1917).

extensive series, however, was that studied at Liverpool by Mr. A. Malins Smith and Mr. J. R. Matthews, who have published two detailed reports on their findings (Smith and Matthews, 1917, 1917 *a*). Taken together, their two series of cases show that, of 450 non-dysenteric patients, 35, or 7·7 per cent., were infected with *E. histolytica*.[1] All the cases were patients in hospitals—200 soldiers invalided for various diseases other than dysentery, and 250 non-dysenteric patients in a civil hospital.

It should be noted here that *E. histolytica* was not, of course, the only intestinal protozoon which was sought and found among non-dysenteric cases. Other amoebae, and all the flagellates which were so frequently encountered in the stools of the dysenteric patients, were also found to be not uncommon: sometimes, indeed, there seemed to be but little difference—if any—between the incidence of intestinal protozoal infections in the two classes of cases. It must be remembered, also, that these cases—whether they were returned as 'dysenteric' or 'non-dysenteric'—were nearly all derived from similar areas. The vast majority of them came from Egypt and Gallipoli; and although the 'non-dysenteric' class consisted of men who were not invalided to England for dysentery, a very large proportion of them, when carefully questioned, were found to have suffered recently from dysentery or diarrhoea or some other intestinal disorder during their service abroad. Nevertheless, there were some 'non-dysenteric' patients—though comparatively few—who were certainly infected with *E. histolytica* and from whom no history of intestinal disorder could be elicited. These men appeared to be true contact carriers of the parasite—as defined by Walker (1913); but how long they had carried their infections, and whether they had acquired them originally abroad or at home, were questions upon which the findings threw no light.

There were, in 1916, two individual cases, well known to all of us working in London, which were of special interest. The first of these was studied and described by Dr. C. M. Wenyon (1916). The patient was 'a labourer in London, who had never been out of England', but who 'had worked on a transport then lying in dock'. He contracted dysentery and was admitted to the London Hospital, where his symptoms disappeared. He remained ill, however, and was found to have a liver abscess, which was opened and drained. In the scrapings from its wall, active *E. histolytica* amoebae were found, and the cysts of the same parasite were found in his stools. The patient died, and at autopsy a second abscess was found in his liver, and a few small amoebic ulcers in his large intestine. This was undoubtedly, therefore, a case of

[1] In their first report, Smith and Matthews (1917) remark (p. 365) that their results—namely, the finding of a considerable percentage of *E. histolytica* infections among soldiers invalided for diseases other than dysentery—were 'confirmed' by mine (Walton Hospital, 1916). This is a somewhat misleading statement, as my results were obtained and published first, and theirs actually supplied a confirmation of mine. The work of Smith and Matthews was, indeed, carried out at my suggestion—as they fully acknowledged (p. 389); and I made the suggestion because of the results that I had already obtained whilst working along similar lines.

E. histolytica infection acquired in England; but at the time it appeared probable—as Wenyon inferred—that the patient had contracted his infection 'from material from some carrier case who had been at the dock or on the transport'.

The second case was one which has been recorded by Dr. G. C. Low and myself (Low and Dobell (1916), Case 3). The subject was a young man who attended my class, in 1916, for instruction in the routine examination of stools, in order to take up the work of diagnosis at a military hospital. Like the other members of the class, he subjected his own stools to careful microscopic examination, and was surprised to find that he was passing cysts of *E. histolytica*—an observation which I was able to confirm. This worker had never at any time suffered from dysentery, persistent diarrhoea, or other intestinal disorder, and displayed no symptoms of intestinal disturbance at the time of the discovery. He had, however, been abroad—to Switzerland, Southern France, and Northern Italy—though never in the tropics or in any places where amoebic disorders were known to be prevalent, and had resided in England for the preceding five years. It seemed probable, at the time, that he had accidentally acquired his infection in the laboratory, in the course of examining stools containing cysts of *E. histolytica*. Two circumstances, however, led me to doubt the correctness of this supposition. In the first place, this worker was particularly careful and cleanly in his work—in fact, so fastidious, owing to his natural repugnance to handling stools, that he seemed the least likely of any member of the whole class to acquire an infection accidentally. The second point was this: he was infected with a race of *E. histolytica* forming cysts of unusually large size,[1] and—so far as I could ascertain at the time—no sample of faeces containing similar cysts had been examined by him whilst he was working with me. I am now inclined to believe that in this case the infection was really not acquired in the laboratory, but that the subject was a contact carrier who had unknowingly been infected for some years at least, and had contracted his infection very probably in the British Isles.

There is one other case which became known at about the same time and which ought, perhaps, to be placed in the same class as the two just mentioned. This is the case recorded by Worster-Drought and Rosewarne (1916). The patient was a soldier who had never left England, but had lived all his life in or near Manchester. He enlisted in 1915, and was admitted to the Royal Herbert Hospital, Woolwich, in the following year, with a typical attack of acute amoebic dysentery. *E. histolytica* was found in his stools; and he recovered rapidly, and apparently completely, as a result of emetine treatment. No carriers of *E. histolytica* could be found among this patient's companions; but whilst it is certain that he was a case of amoebic dysentery

[1] That the cysts were those of *E. histolytica* there can be no question: and confirmation—if it were needed—is supplied by the fact that the infection was entirely eradicated by treatment with emetine bismuth iodide.

acquired in England, the manner in which he became infected is, as in the other cases just mentioned, still uncertain.

At this time it was generally recognized that carriers of *E. histolytica* are by no means rare in the tropics, and probably also in sub-tropical countries. That many soldiers returning from the Near Eastern theatres of war were infected with the parasite had been shown conclusively: and as *E. histolytica* was not known to occur in the normal civil population of Great Britain, it seemed natural to suppose that the returning carriers constituted the source from which were derived the few known cases of infection undoubtedly acquired in this country. A very important point, however, which made one hesitate to accept this conclusion unreservedly was this: *E. histolytica* was known to be disseminated in the same way as the other protozoa of the human bowel—the harmless amoebae and flagellates. These we knew to occur in persons who had never left the British Isles. Why, then, should not *E. histolytica*, if once introduced into this country, spread through the population in precisely the same manner? That there had been abundant opportunity for its introduction in the past could hardly be doubted: and considering this question in all its bearings, I soon came to the conclusion that it would be somewhat remarkable—to say the least—if *E. histolytica* were absent, at the present time, in the native British population. Against this supposition, however, was the fact that typical amoebic diseases—dysentery and liver abscess—appeared to be extremely rare in this country. Yet this objection might not be very cogent: for we were beginning to realize that amoebic diseases are not very common even in countries where *E. histolytica* is notoriously endemic, and that carriers of the parasite, displaying no clinical symptoms of infection, are far commoner than was originally supposed. We knew at that time, with a fair degree of certainty, that the dysenteric convalescents reaching this country from abroad were heavily infected with *E. histolytica*: but we also knew—or were beginning to know—that the dysentery from which these patients had suffered in the field was, in most cases, not amoebic but bacillary. From reliable observations made on the spot it seemed certain that less than 10 per cent. of the dysentery in the armies abroad was due to *E. histolytica*; whereas in the same troops, when they reached England, twice or thrice this percentage were found to be carriers of the parasite.

Another fact also came to light in the winter of 1916–17. Soldiers returning to England from the French front, many of them men who had never at any time been in any countries save England and France, were also found to be infected to a considerable extent with *E. histolytica*. Mr. Redman King, at Barton Dysentery Dépôt, investigated a number of such cases; and in a series of 1,300 convalescent dysenteric patients, whom I examined at this time at the Manor War Hospital, Epsom, I found[1]

[1] See Dobell, Gettings, Jepps, and Stephens (1918).

—after a most careful inquiry into their past histories—that no less than 8·37 per cent. of the men (788 in number) who had been in England and France only, were carriers of *E. histolytica*. Amoebic diseases were not known to be more prevalent, in normal circumstances, in Northern France than in Great Britain; and moreover, it was fairly certain that by far the greater number of the dysentery cases occurring on the French front were bacillary—not amoebic. Consequently, unless infection with *E. histolytica* had spread through our armies from the associated colonial troops—and had spread with remarkable rapidity, and without concomitant clinical manifestations—it seemed certain that carriers of the parasite must be far commoner than had hitherto been supposed in the native population of Great Britain, of France, or of both countries.

The only way to arrive at the truth of the matter was obvious. The civilians—the normal resident population—would have to be subjected to examination in the same way as the military patients. In this way alone could the findings from the latter class be properly interpreted. But this was not easily done. It was difficult to obtain samples of faeces from a large number of persons who had never been abroad; and yet such material was obviously necessary for this investigation. Anybody who had been out of Britain would have to be excluded; for it would be impossible, if protozoa of any sort were found in his stools, to ascertain whether the infection had been acquired at home or during his sojourn in foreign parts: and this meant that a very careful inquiry would have to be made into the history of every case that was to be included in our 'British' series. Then again, it was obviously necessary to examine as large a number of the 'British' population as possible, since a small sample might give a wrong idea of the real conditions. And finally there was the difficulty of obtaining workers for the undertaking. The investigation, if it were to be of any value, would have to be made by competent workers, whose findings could be trusted: and the men and women whom I had trained for this purpose, and upon whose diagnoses reliance could be placed, could ill be spared from the hospitals where they were then employed in the examination of military patients. Their work for the Army naturally took precedence of all other work.

I made several attempts to carry out investigations of this sort myself, but owing to preoccupation with other more urgent duties the results which I obtained were insignificant. But I endeavoured to impress upon all the workers whom I trained from time to time for the War Office the importance of conducting inquiries into this problem; and I urged them all to seize every opportunity of obtaining the information needed. For various reasons the majority of these workers were unable to devote their attention to the matter; but one group of them, acting upon my suggestions, ultimately succeeded in obtaining data of the greatest interest. I refer, of course, to the workers at the Liverpool School of Tropical Medicine, whose results have

now been fully published and are already well known. The work was begun by Mr. A. Malins Smith, Mr. J. R. Matthews and Miss Doris L. Mackinnon, who passed through a course of training with me in London in the early part of 1916, under the scheme arranged by the Medical Research Committee and the Royal Society for the War Office. On going to Liverpool these workers were joined by Mr. H. F. Carter, and received the assistance of Professor Warrington Yorke. Later Mr. Malins Smith and Mr. Matthews continued the work alone, and it is to their efforts that most of our present knowledge is due.

I shall consider this important work, carried out in Liverpool, in greater detail in later chapters, and will here merely note briefly the general results. In a first report (Yorke, Carter, Mackinnon, Matthews, and Malins Smith, 1917), results were recorded of examinations of the stools of 344 persons who had never left Great Britain. Of these, 138 were 'patients, for the most part surgical, in the Liverpool Royal Infirmary; they consisted of both males and females of various ages': the rest (206) 'were healthy young men of about 18 years of age who had recently entered the army and were in training at a camp in the vicinity of Liverpool'. No less than 10 of these (2 in the first group, 8 in the second) were found to be infected with *E. histolytica*—a percentage, for the whole, of 2·9, or roughly 3. This figure was obtained, moreover, as a result of making only a single examination of the stools of each case, so that it certainly represents less than the number actually infected. None of these 10 infected individuals had ever suffered from dysentery. The cysts in their stools were typical in every way; and further evidence that they were really those of *E. histolytica* was obtained by animal experiment—a kitten fed upon cysts from one case developing typical and fatal amoebic dysentery.

Further results were announced in the following year in a Report to the Medical Research Committee (Carter, Matthews, Mackinnon, and Smith, 1918), and at a meeting of the Society of Tropical Medicine and Hygiene (Yorke, 1918); and again in 1919, at a meeting of the British Medical Association (Yorke, 1919). Since then Mr. Malins Smith and Mr. Matthews have published a complete account of all their findings (Matthews and Smith, 1919, 1919 *a*). The five reports published by them summarize the results obtained in the examination of British residents—never abroad—belonging to the following classes: (1) Civilians in an infirmary, (2) Army recruits, (3) Children, (4) Asylum patients, (5) University and school cadets. Infections with *E. histolytica*, ranging from 1·5 per cent. (infirmary patients) to 9·7 per cent. (asylum patients), were found in all these series. Other protozoa were, of course, also found and recorded, but will be considered later in the analysis of the total findings from all sources.

Investigations along somewhat different lines were made during 1917 by Dr. P. P. Laidlaw at Guy's Hospital. He searched for *E. histolytica* in patients with symptoms suggestive of infection

with this parasite in the hope of finding indigenous cases of amoebiasis; and he soon succeeded in finding three such cases (Laidlaw, 1918). The first case was one of chronic amoebic dysentery: the second a case of amoebic abscess of the liver, without concomitant dysentery but with abundant cysts of *E. histolytica* in the stools. Neither of the patients had ever been abroad. Both were males, aged 38 and 48 respectively, admitted to hospital in the usual way—Case 1 for 'ulcerative colitis', Case 2 with 'lumbar and abdominal pain'. The diagnosis of the second case was arrived at only after laparotomy and evacuation of the abscess thereby discovered. Typical specimens of *E. histolytica* were obtained from both cases,[1] and from both kittens were inoculated and successfully infected. Emetine treatment also had a beneficial effect on both patients. The third case reported by Laidlaw was based upon material supplied by Dr. Perdrau, who showed him sections of an intestinal ulcer and a liver abscess found *post mortem* in a woman who had never left Britain. In both these *E. histolytica* was present.

Evidently these were all cases of indigenous amoebiasis, and they would probably all have passed unrecognized if they had not been specially looked for. They were found among the ordinary admissions to a large London hospital in the same year; and from his experience Dr. Laidlaw was led to suggest that, even in Britain, 'cases of this type are not so rare as might be thought from the literature'. He concluded 'that cases of ulcerative colitis, colitis, and liver abscess should always be examined with a view to excluding amoebic infection'.

In the summer of 1918, the War Office Committee on Dysentery,[2] with the results of the Liverpool workers and others before them, decided that further inquiry into the incidence of *E. histolytica* infection among the resident civilian population of Great Britain was desirable. The time was considered opportune to utilize the services of some of the trained workers, then engaged in examining dysenteric convalescents at military hospitals, for this purpose—the pressure of work at these establishments having, at that moment, somewhat abated: and I was asked by the Chairman of the Committee to organize and supervise investigations, with the aid of the personnel then available at various centres, along the lines followed by the workers at Liverpool and with the object of checking and amplifying their findings. Accordingly, in July 1918, I invited five of the protozoologists working at military hospitals to investigate, as far as possible, the protozoal infections occurring in the resident civilian populations in the areas in which they were situated. These workers had all been through a course of instruction with me in London before they were employed at military hospitals; and as they had all, by this time,

[1] Dr. Laidlaw kindly sent me specimens for examination, and I had no difficulty in confirming his diagnosis.

[2] Consisting of Major-General Sir William Leishman, K.C.M.G. (Chairman), Sir Walter Fletcher, K.B.E., Professor (now Sir) F. W. Andrewes, Dr. G. S. Buchanan, C.B., Dr. G. T. Western (Secretary), and the present writer.

had considerable experience in this line of work, it was believed that reliance could be placed upon their findings. These five protozoologists were:

A. H. Campbell, B.Sc., 2nd Southern General Hospital, Bristol.

T. Goodey, D.Sc., 1st and 2nd/1st Southern General Hospitals, Birmingham.

R. C. McLean, M.A., Reading War Hospital.

Miss M. M. Nutt (Bathurst Student, Newnham College, Cambridge), Leeds and Sheffield Military Hospitals.

A. G. Thacker, A.R.C.S., Kitchener Hospital, Brighton.

Mr. Campbell and Mr. Thacker were working under grants from the Medical Research Committee: the three others were in the service of the War Office. They all expressed their willingness to collaborate in the undertaking, and were then supplied with cards for the recording of their findings. These cards, which were issued by the Medical Research Committee, were designed—by myself, in consultation with Sir Walter Fletcher—so as to indicate the kind of information required, and in order to enable the examiners to record their findings, and such essential facts as they could elicit regarding the histories of the cases studied, in a uniform manner and with economy of space and time. The form of the card is shown on p. 14—its actual measurements being 12·5 × 20 cm.—and it may be added that it appears to have fulfilled its objects. Apart from this provision for collecting and correlating the results, all arrangements for the work were left in the hands of the protozoologists themselves. They were all able, with the assistance of the medical officers in charge, and in some instances with aid from the Medical Research Committee and the Local Government Board, to obtain the necessary material from various local institutions.

As all the workers were engaged simultaneously in the routine work of diagnosis for military hospitals, they could devote only a fraction of their time, as opportunities occurred, to the examination of civilians. There were naturally many difficulties to be surmounted—differing from time to time and from place to place—and the several workers did not all enjoy equal facilities for the prosecution of their inquiries. This explains, to a large extent, the inequality of their results. Nevertheless, every worker was able to investigate a certain number of cases; and in due course each was able to submit a report of the results. The largest series of cases—and the fullest report—came from Miss M. M. Nutt (Leeds and Sheffield); but she had already begun her inquiry, at my suggestion, before the matter was taken up by the War Office Committee. The reports are all printed here for the first time (see Chapter III), and deal, in the aggregate, with the findings from the examination of the stools of over one thousand

Examined at

Case No.

Name

Sex

Occupation

Age

Usual Place of Residence

Places of Residence abroad, with dates

Previous attacks of Dysentery

Where?

When?

Persistent Diarrhœa?

Liver Abscess?

PROTOZOOLOGICAL EXAMINATIONS OF STOOLS.

Dates	Findings.

Remarks

British civilians who have never, at any time, been abroad. Nobody who realizes the difficulties involved in such an undertaking will be disposed to complain of the smallness of this number. On the contrary, it will, I think, be generally conceded that to have found and examined so large a number of persons of this class constitutes, when all the circumstances are borne in mind, a performance which does the greatest credit to all the workers concerned.

The investigation was gradually discontinued in 1919 following the Armistice and the disbanding of the Army, and the consequent reorganization of Dysentery Hospitals and Dépôts and the return of the temporary protozoologists to their ordinary civil employments. By the beginning of the present year (1920) the reports from all the centres, together with the cards, duly filled up, were in my hands; and it was then possible to collect, analyse, and classify the results obtained from all sources. This is the main purpose of the present Report.

It should be added here that at the time when the War Office Committee decided to promote these inquiries, a similar investigation had already been begun in the Navy. After the publication of the results obtained by the Liverpool workers, Surgeon Captain P. W. Bassett-Smith, C.B., R.N., urged upon the Admiralty the importance of investigating the new entries to the Royal Navy, in order to ascertain the extent to which they were infected with *E. histolytica* and other intestinal protozoa. Accordingly, arrangements were made by Surgeon Lieutenant-Commander P. Fildes, R.N.V.R., at the Royal Naval Hospital, Haslar, for carrying out this investigation. Examinations were made by Mr. H. A. Baylis —one of the protozoologists previously trained for this work, like the others, by me in London—of 400 healthy men joining the Navy and Marines. Reports on his findings have already been published (Baylis, 1919, 1920), and will be noted in greater detail later. It will suffice to notice here that ten cases of *E. histolytica* infection were found among these men—all healthy individuals, drawn from various parts of the British Isles.

It is not possible to include this series with the others for the purposes of this Report. Particulars of past history were not obtained for every case, as 'it was thought sufficient to make special inquiry as to the history of each case found to be a carrier of *Entamoeba histolytica*'. Consequently, as some of the cases had probably been abroad, they cannot be regarded as forming a series exactly comparable with the others in this respect. A set of cards was sent to Mr. Baylis, but at that time his investigations were already in progress, so that he was only able to enter on them particulars of the cases infected with *E. histolytica*. As Mr. Baylis kindly returned these cards to me when his inquiry was published, I have been able to utilize them in considering his results and comparing them with the rest.

In dealing with the material at my disposal for this Report I have not confined myself to analysing the work that was instituted by the War Office Committee. This work, to be properly

appreciated, must be taken in conjunction with that done previously. The cases examined by the Liverpool workers were more numerous, and really form the backbone of the whole inquiry. The newer findings here presented are supplementary, and the two series must be added together in order that their significance may have a maximal value. Moreover, since no comprehensive treatment of this subject has previously been possible, and since, in consequence, no complete survey of it has been attempted, it has seemed to me opportune to summarize all the information at present available. I have therefore extended the scope of the present Report so as to include the following subjects—to each of which a separate chapter will be devoted:

1. The older records of the occurrence of intestinal protozoa in the population of Britain.

2. The recent reports on the same subject—comprising a summary of the results obtained by the Liverpool workers, and the newer findings of the workers under the War Office Committee (now published here for the first time).

3. A summary of all the results so far obtained, with a discussion of certain points of particular interest.

4. A brief review of certain similar observations recently made in France, Holland, and Germany.

And in a final chapter I will present very briefly what seem to me to be the most important conclusions deducible from the facts recorded in all the antecedent chapters.

Infections with intestinal protozoa are, in general, very persistent; and we know that they are by no means uncommon in many foreign countries. Consequently, it has seemed to me necessary, for present purposes, to investigate particularly the intestinal protozoa of persons who have never been abroad. When such persons are found to be infected, it can be said with certainty that their infections were acquired in Britain. It may be urged, however, that even in such cases the infection may not be truly indigenous, but may have been acquired from some imported foreign source—from a home-coming soldier, for example, returning from a country where the infection is endemic. This objection is, I think, negligible; for even if the explanation were true, it would not invalidate the conclusion that infection can be acquired in this country, and it would demonstrate, moreover, that the conditions here are suitable for such infections to spread—a very important point. For if they can spread now, they could have done so before, and with a fluctuating population like ours there is every reason to suppose that the infection—whatever it may be, and whenever it may have been first imported in the past—would thus long ago have established itself and become as truly 'endemic' as it was in its country of origin.

As regards the present classification of records into 'old' and 'new', I would only remark that it is purely arbitrary, and done for convenience. What we have seen occurring during the War

must have been going on—probably more slowly—for centuries: but it is only recently that attention has been devoted to the subject, and that any considerable body of observations has been amassed. The outbreak of war in 1914 forms a convenient dividing line for my present purposes, and I have therefore called all records prior to this date 'old', and all those since 'new'.

In the next chapter I shall now analyse, as briefly as possible, all the old records which I have been able to find in the literature.

CHAPTER II. THE OLDER RECORDS OF THE OCCURRENCE OF INTESTINAL PROTOZOA IN THE POPULATION OF BRITAIN.

The occurrence of intestinal protozoa in the population of the British Isles—that is, in residents who have never been abroad—has been the subject of serious inquiry only since the outbreak of the War. Consequently almost all that has been definitely ascertained has been the product of the last half-dozen years. Nevertheless, it was known prior to 1914 that amoebic dysentery and liver abscess do occur sporadically in Britain, and several apparently indigenous cases had been recorded. Of such cases I have been able to find but five for which the evidence appears at all convincing. I shall summarize them briefly at this point, as they are of interest in two respects: first, from a historic standpoint; secondly, because they show that *E. histolytica* occurred in the population of Britain before the War—that is, before the recent demonstration of its presence.

Although it was doubtless well known to many people before the War that *E. coli*, *Giardia*, and other non-pathogenic species of intestinal protozoa occur in the resident population of the British Isles, I have been unable to discover any printed records of this in pre-war literature. Consequently this chapter will deal entirely with records relating to the occurrence of *E. histolytica*.

The older evidence for the existence of *E. histolytica* in persons who have never left Britain is, of course, mainly pathological and clinical. But the symptoms and lesions of such infections are very characteristic; so that, notwithstanding the fact that the certain recognition of *E. histolytica* is a comparatively recent achievement, it is possible to draw conclusions from several of the older records with some degree of certainty.

I shall now review, in their historic sequence, all the 'old' records which I have been able to find.

Dickinson's two cases.—The earliest British case of *E. histolytica* infection known to me is that recorded by W. Howship Dickinson (1862). His patient was a woman aged 37, who came under observation at St. George's Hospital. 'She had never been out of England.' She was 'sallow', and complained of 'pain in the right hypochondrium', and a tumour could be felt extending

from the liver area to below the umbilicus. 'The bowels were confined.' She 'had been ill for one year', with 'pain about the region of the liver'; but she did not 'admit that she had ever had any looseness of the bowels'. 'The ordinary symptoms of dysentery were, as far as could be ascertained, altogether absent.' In hospital she was constipated, and died 8 days after admission.

At the *post-mortem* examination a large single abscess, containing about 1 gallon of pus, was found in the right lobe of the liver. 'The upper part of the large intestine was in a state of ragged ulceration, such as occurs in dysentery. It ceased abruptly at the edge of the ileo-caecal valve. The absorbent glands in the neighbourhood were red and swollen.'

Though no amoebae were described in the pus from the abscess or in the ulcers in the gut, the symptoms and lesions recorded are so typical, in every way, that it is hardly possible to doubt that this was a case of *E. histolytica* infection.

Nearly 20 years later, Dickinson (1881) recorded a second case. The patient was a man—a lath-splitter, aged 36—who was also admitted to St. George's Hospital. On admission he was 'febrile', with a 'temperature ranging pretty regularly from about 98° in the morning to 102° in the evening'. He was found to be suffering from severe dysentery, which was followed by a liver abscess. The disease ended fatally '38 days after the first symptom'. Previous to this illness the patient had enjoyed good health, and it is stated that he 'had never left England'.

At the autopsy, 'the large intestine presented from end to end a patchwork of dysenteric ulcers, separated by narrow ridges of thickened mucous membrane. The ulcers were very irregular in size and shape; they were for the most part deep, some exposing the muscular coat of the bowel. . . . The small intestine was healthy. The liver contained a large single abscess which held about four ounces; it lay in the back part of the right lobe near its upper and posterior surface. The shape was irregular, its walls ragged, and without lining membrane.'

After discussing the *post-mortem* findings, Dickinson says: 'I think we have no choice but to look upon the ulceration and the abscess as cause and effect, and upon the portal vein as the connexion.' He thus came very near to what is probably the correct interpretation, for I have no doubt that this also was a case of *E. histolytica* infection. Everything recorded is absolutely typical of amoebic dysentery and liver abscess. It requires only the finding of amoebae in the abscess and the ulcers to make the description complete. Such a discovery, however, could hardly have been expected at the time when Dickinson's observations were recorded.

Moore's case.—In the year in which the preceding case was recorded, another probable case of *E. histolytica* infection was briefly noted by Norman Moore (1881). His patient was a little girl, aged $3\frac{1}{2}$ years, admitted to St. Bartholomew's Hospital, where she died about 5 weeks after admission. 'The child had never been out of London.' She had two abscesses in her liver,

'one in the right lobe as large as a small orange, and one in the left lobe of the size of a filbert.' The abscesses had 'very definite boundaries', and the neighbouring liver-tissue appeared healthy. 'The whole of the large intestine was extensively ulcerated'; and it is further recorded that the child 'had been ill ten months with diarrhoea, and for about two months there were slime, blood, and general symptoms of dysentery'.

Though little else of importance is recorded, it seems highly probable that this was another case of amoebic dysentery and liver abscess—due to *E. histolytica*—in a person who had never left Britain.

The case of Saundby and Miller.—'A case of amoebic dysentery with abscess of the liver in a patient who had never been out of England' was described some years ago by Saundby and Miller (1909) in Birmingham. This was another fatal case. Their patient was a man—a cycle-rim maker who had lived in the Birmingham district all his life. From the clinical description and the recorded *post-mortem* findings, the case appears undoubtedly to have been one of amoebic abscess of the liver, with typical amoebic ulceration of the large bowel. No history of dysentery is recorded, but it is stated that 'four days after admission' the patient 'passed a stool containing a considerable quantity of blood'—which apparently was not examined microscopically—and thereafter he had 'no more diarrhoea'. The liver abscess was 5 in. in diameter, in 'the posterior part' of the liver, and contained 'brownish-red pus and masses of necrosed liver substance'. Amoebae were found in the pus, from which no bacteria were cultivated 'in ordinary media', and similar amoebae were present in the intestinal ulcers. The authors identified them as 'the *Entamoeba histolytica* (Schaudinn)', though unfortunately this organism cannot be recognized in their description and figures. There can be little doubt, however, on other grounds, that this was really a case of *E. histolytica* infection.

Saundby and Miller claimed that theirs was 'the first observation of the kind which has been recorded in Great Britain'; and it appears probable that it was, indeed, the first such case in which amoebae were actually found. They appear to have thought that the patient contracted his infection from some 'imported' case of amoebic dysentery: but they adduced no evidence in support of the suggestion. They concluded that 'the disease, although no doubt imported into this country from time to time, rarely spreads; that it may do so, however, when circumstances favour it, seems to be established by the present case'. The authors could hardly have written this last sentence if they had known all that we now know concerning the occurrence of *E. histolytica* in Britain.

Marshall's case.—This is another comparatively recent case, described by Marshall in 1912. The patient was a ploughman, aged 28, living near Dunbar. He had never been out of Scotland. He was admitted to the Royal Infirmary, Edinburgh, 'suffering from chronic diarrhoea', which had begun about seven weeks

before. His stools were generally 'watery', but also contained blood and mucus; and numerous amoebae—identified as *Entamoeba histolytica*—were present in them. Attempts were made to infect cats with the amoebae, but at the time of writing the result of the experiments was not known. The patient was treated with ipecacuanha, which appears to have benefited him: for though he relapsed after a first course, he was, when the paper was written, 'almost convalescent' after a second.

It is uncertain how this patient acquired his infection. Marshall suspected that he might have got it from a soldier, who had been in India, in the same neighbourhood. But this soldier was not proved to be a carrier of *E. histolytica*. 'Spores' were present in his stools, 'but whether they were the spores of *Entamoeba histolytica* could not be determined by mere microscopic examination'.

It appears probable that Marshall's case was also one of *E. histolytica* infection, though the published evidence is not wholly satisfactory. The author appears to have held peculiar views regarding the life-history of *E. histolytica*; and from his description and figures it is, unfortunately, impossible to identify the organisms with which he was dealing. Corroborative evidence from animal experiments and emetine treatment is also—as already implied—lacking.

There are other cases of possibly indigenous *E. histolytica* infection on record, but as they all appear doubtful as 'British' cases, I shall not consider them here. It is possible, however, that some of them were really indigenous. The case of Bassett-Smith (1900) will serve as an instance of these doubtful cases, and illustrates the difficulties of interpretation. His patient, a petty officer in the Navy, had been abroad, but had never previously suffered from dysentery. More than two years after returning home to Portsmouth he had an attack of dysentery, followed by the formation of abscesses in the liver and lung. The pus from the former was bacteriologically sterile, but contained active forms of 'the amoeba coli'. The disease ultimately proved fatal. It seems clear that this was a case of *E. histolytica* infection; but there is no evidence to show where or when the patient originally became infected. He may have acquired his infection whilst abroad, and have remained a contact carrier of the parasite until his fatal illness developed. On the other hand, in the absence of evidence to the contrary, it is possible that he contracted his infection in England—either before he went abroad or at any time after his return.

As it is impossible to determine the facts with certainty, I have excluded several other doubtful cases, of a similar type, from the present chapter.

CHAPTER III. RECENT REPORTS ON THE OCCURRENCE OF INTESTINAL PROTOZOA IN THE POPULATION OF BRITAIN.

In this chapter I propose to set out the recent records of the occurrence of intestinal protozoa in the British population. The greater part of the chapter will consist of the new reports—now published for the first time—from the investigators working at Birmingham, Brighton, Bristol, Leeds, Sheffield, and Reading. Reference to these investigations has already been made in the Introduction (p. 12). As there noted, they were undertaken, under the direction of the War Office Committee on Dysentery, in order to amplify the observations of the workers at Liverpool. It thus seems proper to preface them with some account of the Liverpool workers' results, which have now been fully published. I shall therefore begin by giving a brief summary of this pioneer work, before passing on to the later records.

I may note here that there are, in addition to the reports given in this chapter, three other recently published records of the occurrence of *E. histolytica* in persons who have never been abroad. (1) Baylis (1919, 1920), as already noted, found 10 men infected among 400 naval cases at Haslar; and of these 8 had never left the British Isles. (2) Miss D. L. Mackinnon (1918) examined 34 'British' cases among patients at University War Hospital, Southampton, and found one *E. histolytica* infection in a man who had never been abroad.[1] (3) Tayler has recorded an infection with *E. histolytica* in 'one man who has never been out of England', examined at Addington Park War Hospital, Croydon. It is stated that he 'probably contracted the infection while employed unloading transports at Southampton Docks', but the evidence for this conclusion is not given. (See Turner and Tayler (1919), p. 249, footnote.) All these cases are of interest, in that they supply further information concerning the occurrence of *E. histolytica* in Britain; but as other essential particulars are not available, I have not been able to include them with the other results recorded in this chapter.

A. The Liverpool Reports.

Four years ago Smith and Matthews (1916) recorded—apparently for the first time—the occurrence of *Giardia intestinalis* (= *Lamblia*) in two men who had never been out of England. Later, in collaboration with others (Yorke, Carter, Mackinnon, Matthews, and Smith, 1917), they reported the presence of *Entamoeba histolytica*, *E. coli*, and *Chilomastix mesnili* also, found during the examination of a series of 344 persons who had never been

[1] This man was 'an R.A.M.C. sergeant who had attended to dysentery patients on hospital trains'. The other infections found in this series were with *E. coli* (5), *E. nana* (3), *Giardia* (6), and *Chilomastix* (1).

abroad. The same workers gave further accounts of their observations later (Carter, Matthews, Mackinnon, and Smith (1918); Yorke (1918, 1919); Matthews and Smith (1919, 1919 *a*); Smith (1919)). The last two reports published by Matthews and Smith in 1919 contain their final results in detail, and it is from these that the following figures have been mainly compiled.

(1) Civilians in Liverpool Royal Infirmary.

At this institution 450 cases—chiefly surgical—were examined. None of the patients had ever been abroad. The series included men, women, and children, selected at random. Only 'a comparatively small number' had been admitted for intestinal ailments. The stools were examined only once apiece for the whole series, and the findings recorded were as follows:

Total No. of cases examined	450
Infected with:	per cent.
Entamoeba histolytica	1·5
E. coli	6.7
Endolimax nana	2·4
Iodamoeba bütschlii	1 case
Giardia intestinalis	6.0
Chilomastix mesnili	1·5

(2) Army Recruits.

More than a thousand young army recruits who had never been out of England were examined in this series. They were stationed near Liverpool, and had been in camp for times ranging from a few days up to five months. Only one examination per case was made, and the total findings were as follows:

Total No. of cases examined	1,098
Infected with:	per cent.
Entamoeba histolytica	5·6
E. coli	18·2
Endolimax nana	5·5
Iodamoeba bütschlii	0·4
Giardia intestinalis	7.0
Chilomastix mesnili	2 cases

(3) Children.

'Material for the investigation was obtained at the Liverpool Infirmary for Children, where all the patients are under 12 years of age. The cases examined were in hospital for various diseases, the great majority being non-intestinal.' None had ever been abroad, and the total findings, based on a single examination per case, were as follows:

Total No. of cases examined	548
Infected with:	per cent.
Entamoeba histolytica	1·8
E. coli	11.1
Endolimax nana	2.7
Iodamoeba bütschlii	1 case
Giardia intestinalis	14.1
Chilomastix mesnili	1·8

(4) Asylum Patients.

Matthews and Smith (1919*a*) examined the stools of 207 patients in the Lancashire County Asylum, Whittingham. The patients were all males, their ages ranging from 17 to 87. As in the other series, only one examination per case was made. None of the patients had ever been abroad. The findings were as follows:

Total No. of cases examined	207
Infected with :	per cent.
Entamoeba histolytica	9.7
E. coli	45.9
Endolimax nana	12.1
Giardia intestinalis	3.4
Chilomastix mesnili	23.2

In a later paper Smith (1919) reported his findings from a larger series of cases examined at the Lancashire County Asylum at Rainhill. His results were as follows:

Total No. of cases examined	504
Infected with :	per cent.
Entamoeba histolytica	4.2
E. coli	21.4
Endolimax nana	3.0
Iodamoeba bütschlii	1.0
Giardia intestinalis	5.0
Chilomastix mesnili	6.7
Trichomonas hominis	0.8

A few of the patients in this series were examined more than once; but the figures given were calculated from the results of only one examination per case—to keep them in line with the others.

It should be noted that this series is not strictly comparable with the others, as it includes a few persons who had been abroad. It is stated that 'only thirty-four of the five hundred and four patients at Rainhill had, so far as could be traced, ever been abroad': but the findings for the 'British' cases alone are not given, and as the individual histories are recorded for very few cases, it is impossible to estimate—from what is published—how far the inclusion of 'foreign' cases may have influenced the results.

(5) University and School Cadets.

A very small series—unfinished—was examined by Matthews and Smith (1919*a*) from the University Platoon in an Officers' Training Corps, and older boys from a secondary school near Liverpool. All were males who had never been abroad—their ages being from 15 to 19 years. Results (one examination per case):

Total No. of cases examined	41
No. infected with :	
Entamoeba histolytica	1
E. coli	11
Endolimax nana	1
Giardia intestinalis	1

The authors say of this series that their original object had been 'to examine a number of youths from a higher social class and from better homes than the Army recruits whom we had previously reported on. We can only say that infections of the various protozoa exist among such persons.'

I shall not attempt to summarize at this point the many other important facts brought out and discussed in the foregoing reports. All who are interested in the subject with which they deal will read the originals. There are, however, several matters which require detailed consideration, and to these I shall return in the next chapter. It will be more profitable, and much more convenient, to consider these points after the newer results obtained by the other workers have been given.

B. The Later Reports.

As already noted in the Introduction, the five investigators working for the War Office Committee on Dysentery (see p. 13) presented, in due course, reports on their work. They also (with one exception) sent me back the cards—fully filled up, for the most part—with which they had been supplied for the purpose of recording their findings. I have thus been able to check the reports by means of the cards, and in many cases I have added relevant details from the latter when they were not given in the reports themselves. I have also freely exercised my editorial privileges in preparing these reports for press: for it has been possible, and, indeed, necessary, to introduce a greater uniformity of treatment of the material than was possible to the writers of individual reports—unacquainted as they were with the work of one another. I have cast all the tables in the same form, and eliminated or simplified a number of complicated ones when this appeared desirable. I have throughout removed everything which appeared to me superfluous, and have endeavoured to present the essentials as concisely as possible. I have also—as I have, indeed, throughout the whole of the present work—silently corrected the nomenclature of the protozoa to which reference is made, when the names employed have not accorded with current zoological usage. With these modifications, the reports now presented are the work of the authors whose names they bear. Remarks or additions of my own—which mostly consist of supplementary information which I have copied from the cards—are distinguished by being enclosed in square brackets.

1. Birmingham Report.

By T. Goodey.

Faeces samples were obtained from civilians undergoing treatment for phthisis at two sanatoria, viz. Salterley Grange Sanatorium, Cheltenham, one of the Birmingham Corporation sanatoria, and Cranham Lodge Sanatorium, near Stroud, a private

sanatorium. Samples were sent in from 101 persons who had never been abroad, and of these, 70 were residents of Birmingham, and the remaining 31 were, for the most part, residents of places in South Wales, Somerset, and Gloucester, and thus representative of a more rural type of population than the 70 from Birmingham. The examinations were made in the Research Laboratory in Agricultural Zoology, Birmingham University.

Altogether 72 males and 29 females were examined. Three samples of faeces from each were examined in all except three cases, two of which were examined twice and the other only once.

Practically all the individuals examined belonged to the artisan classes. *Entamoeba histolytica* cysts were found in the stools of six persons, only one of whom (Case 10) had experienced any previous bowel trouble.

The results in the case of the other intestinal protozoa are set out in the following table, and call for no special remark here:

Total No. of cases examined	101
Protozoa:	No. of cases infected.
Entamoeba histolytica	6
E. coli	28
Endolimax nana	7
Iodamoeba bütschlii	1
Giardia intestinalis	5
Chilomastix mesnili	1

[As the total number of cases examined in this series is 101, it has not been thought necessary to present these results as percentages.]

I am indebted to Dr. Robertson, Medical Officer of Health for the City of Birmingham, for very kindly granting me facilities to obtain stool samples from Salterley Grange Sanatorium. To Dr. E. G. Glover, M.O. in charge of this sanatorium, my very best thanks are due for the great interest which he took in the work both in the collection and dispatch of the samples and in obtaining the previous medical histories of those cases carrying *E. histolytica*. Through his keenness, too, he obtained samples from patients at the Cranham Lodge Sanatorium, Stroud, in the charge of Dr. Arthur H. Hoffman, to whom also my best thanks are due for the interest taken in the work and for the readiness with which he sent information about the carrier cases.

[The following additional particulars concerning the six cases infected with *E. histolytica* are given on the cards returned from Birmingham, and are added here as they contain interesting information.]

Cases infected with E. histolytica.

Case No.	*Sex.*	*Age.*	*Occupation.*	*Place of residence.*
1	M.	15	Fitter's assistant	Birmingham.
10	M.	51	Engraver	Birmingham.
62	M.	22	Fitter	Bristol.
70	M.	18½	Engineer	Chard, Somerset.
82	M.	35	Munition worker	Birmingham.
103	F.	42	Housewife	Birmingham.

[The following information about five of these cases was elicited by Dr. E. G. Glover (Cases 1, 10, 82) and Dr. A. H. Hoffman (Cases 62, 70).]

Case 1.—Left school when 14 years old. Delivered milk for about 3 months: since employed in about 20 different jobs. Never any tools in mouth, and never repaired old clothing, &c. Born in Birmingham, and has not been elsewhere save for one short visit to a Shropshire village (where he drank tap-water), and one to Bournemouth. Bowels regular as a rule: never constipated. Had diarrhoea lasting one day in 1917, but never passed blood. Has occasional flatulence after heavy meals. No abdominal discomfort. Father a Birmingham man, who died (*aet.* 45) of pneumonia. Mother a Birmingham woman. One brother consumptive, no bowel trouble. No record of dysentery in family. Has never been in contact with foreigners.

Case 10.—Born in Birmingham, where he has lived all his life. Only away on holidays. In engraving industry (steel, gold, &c.) since age of 13—not dusty work. Occasionally holds steel tools in mouth, and does jewellery repairs for private houses. Water-supply always municipal. Healthy boyhood: after 19 years chest troubles. Pleurisy at 22. *Bowels*: seemingly normal till 19, when he had an attack of diarrhoea lasting 2 weeks in midsummer. Free until following summer, when it recurred. Occasional attacks till age of 40, when he went to hospital and had 'draughts'. Since then well to Christmas 1917, when he had diarrhoea for a week, arrested by treatment. Has haemorrhoids, and is rather constipated as a rule. No special abdominal pain during either constipation or diarrhoea, except colic. *Liver* normal: no jaundice. Wife healthy, never abroad.

Case 62.—Born at Brislington, Bristol, where he has lived all his life. Drinks tap-water. Has been a fitter for 8 years. Does not put tools in mouth, and does not repair old clothes or personal articles. Has had diarrhoea very occasionally, but not lately. No blood or slime in stools. No members of family or household with bowel troubles. No foreigners have lived in his house.

Case 70.—Born in Chard, Somerset, and has lived there all his life. Drinks well-water. In present employment for 1 year. Does not hold tools in mouth. Has not repaired old clothes or personal articles. Has not suffered from diarrhoea or any bowel trouble, nor has any member of his household. No foreigners have lived in his house.

Case 82.—Born in Birmingham, and has lived there all his life: never absent on holiday for more than a few days. Has always drunk tap-water. Cabinet maker and upholsterer from age of 14 to 17—chiefly doing repairs from public-houses, smoke-rooms, and private houses (never bedrooms)—and used to put tacks in his mouth. Polisher (metal) from age of 17 to 32. Joined army in 1914. In training in England September 1914 to February 1915: then discharged with septic poisoning, &c. Usual sanitary arrangements in army: in billets—cottages,

&c.—and drank well-water for 2 months. Not in contact with dysentery cases, and never had diarrhoea himself. At age of 3–4 years he had looseness of bowels (hearsay evidence of parents): since then no diarrhoea. But 6 years ago he had so-called 'catarrh of the stomach' (? due to eating pork), which lasted one week, with diarrhoea and abdominal pain—no blood or mucus or straining—and has been quite well since. No history of diarrhoea in parents, wife, or family. Never in contact with foreigners.

[The following further information regarding the infections of the 6 positive cases has been compiled from the cards.]

Case No. 1.—Cysts of *E. histolytica* present in stools in fair numbers. Diameter 9·5 μ to 12·5 μ (in fixed and stained condition). No other protozoa found.

No. 10.—Cysts in moderate numbers. Diameter 10 μ to 12·7 μ, in stained preparation. No other protozoa found.

No. 62.—Cysts in moderate numbers, 12–13·8 μ in diameter. Large numbers of *Giardia* cysts also present.

No. 70.—Cysts in moderate numbers, 10–12 μ in diameter. No other protozoa found.

No. 82.—Cysts in moderate numbers, 12·5 μ to 14·8 μ in diameter. Cysts of *E. coli* and *Giardia* also present in stools.

No. 103.—Cysts in fair numbers. Diameter 10·7 μ to 12·2 μ. No other protozoa found. Stool examined only once.

2. Brighton Report.

By A. G. Thacker.

A small number of microscopic examinations of faeces of civilians were carried out at the Central Laboratory, Kitchener Hospital, Brighton, in August 1918 and again in June 1919. Sixty-nine persons were examined in all. Most of these persons were inmates of the Workhouse Infirmary, but a few were patients in the Borough Sanatorium. None of the sixty-nine persons concerned were suffering from any intestinal complaint.

The ages of the persons varied from nine years to over seventy, and the sexes were almost equally divided. Forty-six cases were examined once only, sixteen were examined twice, three were examined three times, and the other four more often. The intestinal protozoa were found during these examinations in the following numbers:

Entamoeba histolytica	2 cases
E. coli	15 ,,
Endolimax nana	4 ,,
Giardia intestinalis	2 ,,
Chilomastix mesnili	4 ,,

Certain particulars were obtained relating to the lives of the persons concerned, and it was found that fourteen of the persons had been abroad at one time or another, most of them to tropical countries. Thus, one of the persons having *Entamoeba histolytica*

was the wife of a soldier and had been to India with her husband, and had suffered from dysentery whilst there. The other person carrying *Entamoeba histolytica* had never been abroad and had never suffered from dysentery. He had been a butcher, but had been in the workhouse infirmary about six years.

In the fifty-five persons who had never been abroad the intestinal protozoa were found in the following numbers:

Entamoeba histolytica	1 case
E. coli	10 cases
Endolimax nana	4 ,,
Giardia intestinalis	2 ,,
Chilomastix mesnili	3 ,,

My thanks are due to Captain J. R. Dickson, R.A.M.C., officer in charge of the Central Laboratory, for kindly arranging for the work to be done under his supervision; and also to Dr. Forbes, Medical Officer of Health for Brighton, to whom we were indebted for the supply of specimens.

[In addition to the foregoing brief report, Mr. Thacker has sent an account of two military patients investigated by him at Brighton. These cases were studied with great care, and their histories inquired into in considerable detail; but as both of them had been abroad, they could not be included in the 'British' series. As the evidence shows, both were cases of typical amoebic dysentery: but the evidence shows, further, that they probably contracted this disease in England. There are, moreover, indications of the way in which they may have acquired their infections with *E. histolytica*. So little is known at present about cases of English amoebic dysentery, and about the manner of acquiring infection with the parasite, that no excuse seems needed for publishing these two cases here in some detail.]

Notes on two cases of amoebic dysentery probably acquired in England.

Case 1.—This patient's father was in the army. During the Egyptian campaign of 1882 he had a severe attack of dysentery. He never recovered from this dysentery, but had repeated relapses, and died in 1890 of dysentery and Bright's disease. The present patient, who was born in 1884, developed dysentery when a small child (about 2 years old), and it was surmised that he had 'caught it from his father'. He did not have it very severely; but in the acute stage he passed about 20 stools *per diem* with much pain in the region of the colon. After this, all through his life, he had periodic severe relapses, four to six times a year. He had 15 to 20 stools *per diem* in these relapses, with severe pain. The patient states that he used sometimes to see blood in his stools during these attacks, but as a rule the bleeding was not profuse. He grew up generally delicate, and became a boot repairer. He joined the army in 1915, and while in the army had several of his usual rather mild

attacks of dysentery. He had a more severe attack at Albert in December 1916—about 25 stools *per diem*, with blood and slime. He was in hospital at Abbeville for nearly six months, and was treated with emetine. After rejoining his unit in May 1917, he had two more mild attacks, and then came home on leave in November 1917. Whilst in England (his home is in Brighton) he entered hospital for ulcers on his legs, and whilst in hospital had another attack of dysentery and was transferred to the 2nd Eastern General Hospital, Brighton, where I first saw him (March 1918). *E. histolytica* was found in his stools at the first examination. He was treated first with emetine injections (1 grain a day for 12 days), but remained positive during the course, and cysts were afterwards found in his stools in great numbers. He was then treated with emetine bismuth iodide (3 grains daily for 12 days); but he again remained positive during the course, and numerous cysts were passed afterwards. As this treatment made him very ill, he was next treated with 'Chaparro'—the usual 10-day course. Ten subsequent negative examinations made over a period of nearly a month allowed him to be passed on to the Dysentery Dépôt at Barton as 'negative'. Here, however, he was again found positive at the third examination. He was then treated twice with emetine bismuth iodide (at Barton)—each course lasting 16 days. After his second course he was passed out of Barton as 'cured'. (He forgets exactly how many negative examinations were made.)

This patient was infected with *E. coli*, *E. nana*, and *Trichocephalus*, in addition to *E. histolytica*. Save for his service in France in the army, he had never at any time been abroad.

Case 2.—This patient had never had dysentery or even severe diarrhoea before the War. He had, however, travelled a lot in Europe—France, Belgium, Holland, Germany, and Spain. (Only once in Spain, in 1902: never further south than Madrid.) He joined the army in September 1916, when 39 years old, and went to Aldershot about November 20, 1916. In Aldershot he shared a room with five other men, one of whom had been in Mesopotamia, and the other four in India. Several of these had had dysentery out East. Patient's battery and another battery used the same latrine; and about December 10 a dispute arose between the two batteries in regard to the cleaning of this joint latrine, with the result that neither battery would arrange for the cleaning to be done. The latrine remained in a dirty state for several days. On December 13 my patient had a sudden attack of dysentery. He was due to go on leave on December 15, so did not report sick, but went home to Halifax. After he reached home he got rapidly much worse, but was treated by his own doctor and had no laboratory diagnosis. He passed about 20 stools daily with much blood. Returned to Aldershot in February 1917 and subsequently went to France, where he had periodic severe attacks of diarrhoea. He was invalided for trench fever in November 1917, and sent to various hospitals. In January 1918 he went into training again at Shoreham. His diarrhoea got worse, and he was invalided

into the 2nd Eastern General Hospital as an 'Enteritis' case in May 1918. I found him positive for *E. histolytica* on the first test. He was then transferred to the Kitchener Hospital, and treated first with emetine injections (1 gr. daily for 12 days). This failed. He was then treated with emetine bismuth iodide (usual 12-day course, 3 gr. *per diem*). This apparently succeeded. Ten negative tests were made by me over a period of six weeks following treatment, and he then passed successfully through Barton Dépôt as a 'negative' case.

3. Bristol Report.

By A. H. Campbell.

The material for examination was obtained from Stapleton Workhouse, Bristol, and the Children's Hospital, St. Michael's Hill, Bristol. The stools were examined in saline or iodine solution in the usual way, and if necessary fixed and stained preparations were also made.

This work would have been impossible without the very careful collection of material by Dr. W. R. Norgate at the Stapleton Workhouse and the Medical Officers at the Children's Hospital. I am much indebted to them for their assistance, and also to Professor Walker Hall, M.D., for making the general arrangements and for continual interest and advice. The work was carried out in the Pathological Laboratory of the University of Bristol.

Altogether, the stools of 213 adults (including all persons over 12 years of age) and 49 children under 12 were examined. Repeated examination of the same case was not always possible—owing to death or discharge from hospital. The number of examinations made per case was as follows:

	Adults (over 12).	*Children (under 12).*
One examination . . .	22	41
Two examinations . . .	54	2
Three examinations . . .	137	6
Total No. examined . . .	213	49

[It was found on inquiry that 31 inmates of the workhouse had been abroad, whilst particulars of 4 others were unobtainable. If these 35 cases are eliminated from the series, the total number of persons who had never been out of Britain was 227 (178 adults, 49 children). The protozoological findings in these 227 cases are shown in the following table.]

Persons who have never been abroad.

Total No. of cases examined 227

Protozoa:	No. infected.	Percentage.
Entamoeba histolytica . . .	7	3.08
Entamoeba coli	105	46.2
Endolimax nana . . .	15	6.6
Giardia intestinalis . . .	15	6.6
Chilomastix mesnili . . .	24	0.5

[Although the incidence of *E. histolytica* infection in the adults and in the children was not very different, when the small numbers examined are taken into account, there was a notable difference in the two groups with regard to the other protozoa. This will be evident from the following table.]

	Adults.	Children.
Total No. examined	178	49
Percent. infected with :		
E. histolytica	3·3	2·0
E. coli	53·9	18·3
E. nana	7·8	2·0
G. intestinalis	3·9	16·3
Ch. mesnili	13·4	0·0

[The Stapleton Workhouse series included a number of mental cases (68 in all). The figures obtained for all of the inmates of this institution (213) were:

	Mental.	Non-mental.
Percent. infected with *E. histolytica* . .	2·94	2·75

It is thus evident that the 'mental' and 'non-mental' cases were not appreciably different, so far as their degree of infection with *E. histolytica* was concerned.]

[The following are particulars—compiled from the cards—of the 7 persons who had never been abroad and in whose stools cysts of *E. histolytica* were found.]

Case No.	*Sex.*	*Age.*	*Occupation.*	*Other protozoa found.*
32	M.	52	Porter.	Nil.
78	M.	74	Sanitary worker.	*E. coli.*
103	M.	65	Lithographic writer.	*E. coli* ; *Chilomastix.*
114	M.	3	Nil (infant).	*E. coli* ; *E. nana.*
198	F.	44	Nil (mental case).	*E. nana.*
207	F.	49	Nil (mental case).	*E. coli* ; *Chilomastix.*
237 a	M.	14	Nil.	*E. coli.*

[Case No. 32 had recently had an attack of typhoid, whilst No. 78 is stated to have suffered from dysentery 6 years before. The remainder gave no history of dysentery, diarrhoea, or other intestinal ailment.]

In all these cases *E. histolytica* was found in the encysted condition in the stools. The cysts were all of races ranging from 11·5 μ to 12·8 μ in average diameter. None of the races of *E. histolytica* with smaller cysts were met with.

Out of the whole series of cases (213 adults, 49 children), 22 (all adults) had a previous history of dysentery. Only one of these was found infected with *E. histolytica*. But 50 adults and 16 children had suffered at some time from chronic diarrhoea, and in none of these were the cysts of the parasite detected.

4. Leeds and Sheffield Reports.

By Muriel M. Nutt.

The following are the results obtained from the examination of the stools of civilians with a view to ascertaining what percentage of the normal population of this country is infected

with *Entamoeba histolytica* and other intestinal protozoa. The majority of the examinations were done at Leeds on specimens obtained from patients at the General Infirmary and Workhouse Infirmary. A number of specimens have also been obtained from the Royal Hospital, Sheffield.

Acknowledgements.—I am extremely indebted to Professor M. J. Stewart for the trouble he took in getting me specimens from the Leeds General Infirmary, and for his kindness and the interest he took in this work. I also wish to thank Dr. Allen of the Leeds Workhouse Infirmary for allowing me to obtain specimens from the workhouse patients, and Major Jamieson for the facilities he gave in starting the work at the East Leeds War Hospital. To Dr. Gruner I am indebted for the specimens from the non-dysenteric military patients at Killingbeck and East Leeds War Hospitals (see Appendix, p. 36). At Sheffield my work at the University has been greatly facilitated by Professor Sholto Douglas's kindness and help. I must also express my gratitude to Dr. Hall for his kind assistance in obtaining specimens for me from the Royal Hospital, and to Dr. Naish for those obtained from the Children's Ward of the same Hospital.

Technique.—The technique employed has been exactly similar to that used in the routine examination of the military cases. Two preparations were made from every specimen, from different parts of the stool, the preparations being made in normal saline or Weigert's iodine solution.

(*a*) *Leeds.*

Two classes of civilians have been examined—adults, and children under twelve years old. The following table gives the percentages of protozoa found in the total number of persons examined:

Total No. of persons examined	461	
Percent. infected with :	*Adults* (333).	*Children* (128).
Entamoeba histolytica	1·2	1·6
E. coli	21·0	13·3
Endolimax nana . . .	2·4	4·7
Iodamoeba bütschlii . . .	0·3	0·0
Giardia intestinalis . . .	3·6	39·8
Chilomastix mesnili . . .	11·4	7·8
Trichomonas hominis . . .	0·3	0·0

It was necessary to find out how many of the 461 civilians examined had ever been abroad. In the next table, therefore, these are divided into different groups, A, B, C. In *Group A* none of the 210 adults had ever been out of this country, and of these 3 were infected with *E. histolytica*. All the 128 children examined were found to belong to Group A, and of these 2 were passing *E. histolytica* cysts. *Group B* consists of those persons of whom it was impossible to ascertain with certainty whether they had ever been abroad. As far as could be gathered from the sisters in the wards and other indirect sources it seemed very

unlikely, but as information could not be obtained from the patients themselves it seemed best to place them in a separate class as uncertain. In *Group C* are placed 31 cases who have been abroad at some period of life. One out of these was positive for *E. histolytica*. The small number of persons in this group necessarily makes the percentage of positives higher here than in the other two:

	Group A.			
	Adults.	*Children.*	*Group B.*	*Group C.*
Percent. infected with:	(210)	(128)	(92)	(31)
Entamoeba histolytica	1·4	1·6	0·0	3·2
E. coli	21·9	13·3	22·8	9·7
Endolimax nana	2·9	4·7	1·1	3·2
Iodamoeba bütschlii	0·0	0·0	1·1	0·0
Giardia intestinalis	3·8	39·8	4·3	0·0
Chilomastix mesnili	12·4	7·8	8·7	12·9
Trichomonas hominis	0·0	0·0	1·1	0·0

Notes on the Cases infected with E. histolytica.

Case I. 1. Woman of 69. Has never been out of the country. Was formerly in a mill, now does charing. Place of residence, Leeds. Has had occasional attacks of diarrhoea, both lately and in the past. This was a case showing precystic (‘*minuta*’) amoebae, average diameter 12–13 μ. (Also infected with *E. coli* and *Giardia*.)

Case I. 2. Man of 67. Never been out of this country. Residence, Castleford. Never troubled with diarrhoea or any other intestinal complaint. Cysts few, with an average diameter of 13 μ. (Also infected with *E. coli*.)

Case W. 3. Man of 66. This man was paralysed and had great difficulty in speaking and making himself understood, but as far as could be gathered it seemed very unlikely that he had ever been abroad. Occupation, gardener. Residence, Leeds. No history of dysentery or diarrhoea. Cysts few, 12–13 μ in diameter. (Also infected with *E. coli*.)

Case I. 4. Woman of 72. Fifty years ago had been in New York. Used to take in sewing as an occupation. Residence, Leeds. Never had any trouble with diarrhoea nor a day’s illness in her life until she entered the Infirmary for eye treatment. Cysts few, average diameter 7–8 μ. (No other protozoa found.)

Case I. 334. Boy of 11. Has never himself been abroad, but parents are Russian Jews. Never troubled with diarrhoea, and seemed quite healthy and sturdy. Was in the Infirmary for treatment of his eyes. Cysts few, average diameter 12 μ. (Also infected with *E. coli*, *E. nana*, and *Trichuris*.)

Case W. 335. Girl of 10. Tubercular. Never been out of the country. Had no trouble with diarrhoea. Residence, Leeds. Parents have never been abroad. Cysts few, 13 μ in diameter. (Also infected with *E. coli*, *Giardia*, and *Chilomastix*.)

Number of examinations.

The majority of the cases could be examined only once, but a certain number were examined two or more times. Three out of the four adults who were positive for *E. histolytica* were discovered at the first examination, and one at the third examination. Among the children, both positives were found at the first examination. The following table shows the number of examinations made per case for the whole series:

	Adults.	*Children.*
No. examined once	275	103
,, ,, twice	39	20
,, ,, 3 times	17	4
,, ,, 4 ,,	1	1
,, ,, 7 ,,	1	0
	333	128

Sex and Protozoal Infection.

In the following table all the adults examined (333) are divided into males and females, so that a comparison between the incidence of protozoal infections in the two sexes can be made. It will be seen, however, that there is very little difference between them in this respect:

	Adults.	
	Males.	*Females.*
Total No. examined	172	161
Percent. infected with:		
E. histolytica	1·2	1·2
E. coli	22·5	20·5
E. nana	2·9	1·9
I. bütschlii	0·6	0·0
G. intestinalis	3·5	3·7
Ch. mesnili	10·5	12·4
T. hominis	0·0	0·6

Age, Occupation, Residence.

From the above tables age seems to have little or no bearing upon the protozoal infection except possibly in one case, that of infection with lamblia (*Giardia intestinalis*). Children appear to be infected in greater numbers than adults. This is shown in the first table, where for children the percentage is 39·8, while for adults it is 3·8 only. Two groups of children have been examined, and in each group the lamblia infection is higher among them than among adults taken from the same institution. The higher percentage was found among the Workhouse children, where it reached 48·8 per cent., as against 3·6 per cent. found in adults from that institution. Among the children examined from the Leeds General Infirmary the percentage was 23·9 compared with 3·9 found in the corresponding adults.

The age at which children were found to be infected in the greatest numbers was between one and four years.

There does not seem any relation between occupation and infection, neither does the place of residence, whether in a large

town or small country village, seem to have any connexion with the protozoal infection. It should be noted, however, that the majority of the cases examined at Leeds are resident in the poorer parts of the city.

(*b*) *Sheffield.*

From January to May, 1919, I examined the stools of 174 civilians at Sheffield. Of these, 117 were adults and 57 children—all inmates of Sheffield Royal Hospital and Royal Infirmary. On investigation it was found that 6 of the adults had been abroad. These have been eliminated from the series, so that the results summarized in the following table were obtained from 168 cases (111 adults, 57 children) who had never left England. Of these cases, 84 adults and 39 children were examined once only—the remainder twice, thrice, or more often (only 5 cases more than 3 times). The table corresponds to that given for the Leeds civilians, and shows the percentages of the various protozoa found:

Total No. of persons examined	168	
Percent. infected with:	*Adults* (111)	*Children* (57)
Entamoeba histolytica	1·8	5·3
E. coli	26·1	24·6
Endolimax nana	6·3	7·0
Giardia intestinalis	7·2	15·8
Chilomastix mesnili	5·4	5·3

No infections with *Iodamoeba* or *Trichomonas* were found in this series.

[It may be noted, however, that several infections with *Trichuris* were found at both Leeds and Sheffield. The total number of persons found to be passing ova of this worm in their stools was 5, or approximately 1 per cent. of the whole series of 506 persons who had never been abroad.]

The total number of persons found to be infected with *E. histolytica* was 5 (2 adults, 3 children). Below are further notes on these positive cases—none of whom had, of course, ever been out of the British Isles.

Case 1.—Boy of 17. Has never suffered from dysentery or persistent diarrhoea. Residence, Sheffield. Occupation connected with grinding stone. Cysts few, diameter 12–13 μ. (Also infected with *E. coli* and *Giardia*.)

Case 2.—Man of 30. Butcher, residing in Sheffield. Has never suffered from dysentery, diarrhoea, or any other intestinal trouble. Cysts few, 12–13 μ in diameter. (No other protozoa found.)

Case 3.—Boy of 13, living in Sheffield. Has never suffered from dysentery, but occasionally has slight diarrhoea—never severe. Cysts *ca.* 12 μ in diameter, few. (Also infected with *E. coli* and *Giardia*.)

Case 4.—Girl of 8, living at Conisborough. Has never been troubled with dysentery or diarrhoea; but whilst in hospital had

bowels moved 2–3 times a day. In hospital for tuberculosis of knee. Father a soldier, in France. Cysts few, *ca.* 12 μ in diameter. (Also infected with *E. coli* and *Giardia.*)

Case 5.—Boy of 9, living in Sheffield. Has never suffered from dysentery or diarrhoea—and appears perfectly healthy. In hospital for amputation of leg. Cysts few, belonging to two different races—*ca.* 7 μ and 12 μ in diameter. (Also infected with *E. coli.*)

Summary and Conclusions.

At Leeds and Sheffield I examined altogether 506 members of the civil population who had never been abroad. These consisted of 321 adults and 185 children. Out of this number 10 individuals were found to be infected with *Entamoeba histolytica*—that is, approximately 2 per cent. A higher rate of infection was found in children than in adults, the figures being:

5 out of 185, or 2·7 per cent. of children infected with *E. histolytica.*
5 ,, ,, 321, ,, 1·5 ,, ,, adults ,, ,, ,, ,,

The other intestinal protozoa were also found more frequently in children than in adults. At Leeds 32·7 per cent. of adults and 50·8 per cent. of children were found infected with protozoa other than *E. histolytica.* The most striking difference was in the rate of infection with lamblia (*Giardia intestinalis*), the figures being:

	Leeds.		*Sheffield.*	
	Adults.	Children.	Adults.	Children.
Percentage .	3·8	39·8	7·2	15·8

The percentages given are all probably too low, since in the majority of cases they are calculated on the result of one examination only.

Appendix.

Non-dysenteric military patients.

In order that a comparison might be made with the foregoing results obtained from civilians, a certain number of non-dysenteric military patients were examined during July 1918. The majority were nephritis cases at Killingbeck Military Hospital, Leeds, the remainder being surgical cases at East Leeds War Hospital, and about five were from the Leeds General Infirmary. The results are tabulated below:

Total No. of cases examined	71
Protozoa :	Percent. infected.
Entamoeba histolytica	7·0
Entamoeba coli	40·8
Endolimax nana	11·3
Iodamoeba bütschlii	4·2
Giardia intestinalis	8·5
Chilomastix mesnili	4·2

Notes on the cases carrying E. histolytica.

Case M. 462, had been in France 2½ years but never in the East. Had no history of dysentery or diarrhoea or any other intestinal trouble. Surgical case. Cysts 7 μ in diameter.

Case M. 463, had been in France, South Africa, Ceylon, Singapore, but never had dysentery, diarrhoea, or any other intestinal ailment. Nephritis case. Cysts 7–8 μ in diameter.

Case M. 464, had been in France but not in the East. Slight diarrhoea in France about a year ago. Nephritis case. Cysts 10 μ.

Case M. 465. Trench fever case. Unfortunately, this patient was sent away before any information could be obtained from him. Had certainly been in France. Cysts 12 μ.

Case I. 466. Discharged soldier in Leeds General Infirmary. Had been in France but not in the East. Never had a day's illness in his life before he was wounded in France in 1917. The majority of the men in his regiment in France had suffered from dysentery. Cysts 12 μ.

Thus it appears as if about 7 per cent. of the military patients who are sent to hospitals for diseases other than dysentery are carriers of *Entamoeba histolytica*.

5. Reading Report.

By R. C. McLean.

The following examinations were carried out in the laboratories attached to the Royal Berkshire Hospital and the Reading War Hospital.

Those examined were, with few exceptions, inmates of either the Royal Berkshire Hospital or of the Park Isolation Hospital, Reading. I wish to acknowledge my indebtedness to the medical staff of those institutions for permission to conduct the investigation, and to the Matrons and Sisters for their kind help in collecting and forwarding specimens for examination.

The total number of persons examined was 140, divided thus:

Adults { Males 57. / Females 60.

Children under twelve 23.

Of these, 20 (15 males, 5 females) gave particulars of residence abroad. Among these were 7 ex-soldiers with a record of service in France during the War. None of them had dysentery recorded in their medical histories. [The total number of persons who had never been abroad was thus 120. The stools of each were examined only once.]

The total number giving an account of previous intestinal disorder was 4, of whom the following are the particulars:

M. 58. Carcinoma of rectum in July 1918. No residence abroad. No protozoa.

M. 81. Dysentery in Reading 1896. Visited China 1850. No protozoa.

F. 26. Dysentery in Reading 1913—'slight.' Visited the West Indies 1912. No protozoa.

F. 115. 'Clinical Dysentery' at date of examination. A military nursing sister. No residence abroad. No protozoa.

Below is a summary, in tabular form, of the results of the protozoological examinations of the stools of the persons who had never been abroad.

Total No. of cases examined							120
Protozoa:				No. of cases infected.			Percentage.
Entamoeba histolytica	.	.	.	3	.	.	2·5
Entamoeba coli	.	.	.	20	.	.	16·6
Endolimax nana	.	.	.	10	.	.	8·3
Giardia intestinalis	.	.	.	13	.	.	10·8
Chilomastix mesnili	.	.	.	1	.	.	0·8

It is interesting to note that the three cases in which cysts of *E. histolytica* were found were all females in the same ward of the Berkshire Hospital. Their ages and occupations were:

Case No.	*Age.*	*Occupation.*
6	66	Nil (house duties only).
15	19	Domestic servant.
38	18	Laundry maid.

As two of these—Nos. 15 and 38—were young girls in employment in villages, they may be regarded as liable to contact with returned soldiers. The third was a woman living in a good residential district of Reading. None had any trace of intestinal disorder present or past, but they were all three evacuating the cysts in large numbers.

[From the entries on the cards, it appears that the cysts of Case 6 measured 9–13 μ in diameter; those of Case 15, 8–11 μ. The diameter of the cysts of Case 38 is not recorded. It may be added that Cases 15 and 38 were also infected with *E. nana*, and Case 6 with *Giardia*.]

Finally, it may be noted that worm eggs were found in the stools of 11 cases (*Trichuris trichiura*, 7; *Ascaris lumbricoides*, 3; *Oxyuris vermicularis*, 1).

CHAPTER IV. SUMMARY OF ALL REPORTS, AND DISCUSSION OF CERTAIN POINTS OF PARTICULAR INTEREST.

In this chapter I propose to combine and discuss the results obtained by all the workers concerned in the present investigation. I shall begin with a consideration of the incidence of infection with the various protozoa studied.

A. The Incidence of Protozoal Infections in Persons who have never left Britain.

There is now a fairly large accumulation of statistics bearing on this subject, and it is therefore now possible to estimate the probable incidence of several different protozoal infections with some precision. In order to arrive at a just estimate of the results as a whole it will be necessary, in the first place, to add together the findings recorded in the several Reports.

The findings of Smith and Matthews are mostly presented in the form of percentages; and I have therefore worked out their actual figures in order to be able to combine them with those of the later workers. I have thought it necessary, for the present purpose, to exclude the results recorded by them for asylum patients. The people of this category are in several ways not fair samples of the population, and there is reason to suppose that their habits and mode of life are conducive to the acquisition of intestinal infections to an abnormal extent. Excluding, therefore, the mental cases examined by Matthews and Smith (1919*a*) and Smith (1919), I have obtained the following figures from all the Reports published by the Liverpool workers:

Cases.	Total No.	Protozoa[1]					
		E. h.	*E. c.*	*E. n.*	*I. b.*	*G. i.*	*Ch. m.*
Civilians, Royal Infirmary .	450	7	30	11	1	27	7
Army Recruits . . .	1098	62	200	60	5	77	2
Children	548	10	61	15	1	77	10
Cadets	41	1	11	1	0	1	0
Grand total .	2137	80	302	87	7	182	19

This table gives the actual numbers of persons found infected with intestinal protozoa in the four series of cases investigated by

[1] In this and the two subsequent tables the following abbreviations are used for the names of the intestinal protozoa which come into consideration:

E. h. = *Entamœba histolytica.*
E. c. = *Entamœba coli.*
E. n. = *Endolimax nana.*
I. b. = *Iodamœba bütschlii.*
G. i. = *Giardia intestinalis.*
Ch. m. = *Chilomastix mesnili.*

Matthews and Smith: but it must be remembered that the findings are based throughout on the results of only one examination per case. None of the 2,137 persons in the whole series had ever been abroad.

If we now take the results of the newer Reports, given on earlier pages, and express them in the same way—converting percentages into actual numbers as before, when necessary—we obtain the figures shown in the following table:

Cases at	Total No.	Protozoa.					
		E. h.	*E. c.*	*E. n.*	*I. b.*	*G. i.*	*Ch. m.*
Birmingham . .	101	6	28	7	1	5	1
Brighton . . .	55	1	10	4	0	2	3
Bristol . . .	227	7	105	15	0	15	24
Leeds and Sheffield .	506	10	106	23	0	76	45
Reading . . .	120	3	20	10	0	13	1
Grand total .	1009	27	269	59	1	111	74

If we add these findings from 1,009 individuals who have never been abroad to those from the 2,137 similar cases in the Liverpool series, we obtain the following results:

Cases.	Total No.	Protozoa.					
		E. h.	*E. c.*	*E. n.*	*I. b.*	*G. i.*	*Ch. m.*
Liverpool (4 series) .	2137	80	302	87	7	182	19
Six new series . .	1009	27	269	59	1	111	74
Grand total .	3146	107	571	146	8	293	93
Percent.		3·4	18·1	4·6	0·25	9·3	2·9

These final percentages, based on the records obtained from the examination of over three thousand individuals—men, women, and children—who have lived all their lives in Britain, give us material for making an approximately correct estimate of the extent to which the indigenous population of these islands is infected with intestinal protozoa. But they cannot be accepted, as they stand, as correct; and for the following reasons.

The greater part of the series (over 2,000 persons) is composed of the Liverpool cases. These were all examined but once apiece, and they thus form—in this respect—a homogeneous series. The other series, however (over 1,000 cases, or roughly one-third of the whole), consist of persons examined a variable number of times each, and consequently the whole series is not homogeneous in respect to the number of examinations made per case. It is well known—from the figures already collected and published by myself (1917) and others—that the number of infections found in any series examined depends upon several factors: and one of the most important of these is the number of examinations made per case in the series. (I leave out of account now the other factors—such as the competence of the examiner, the time taken in making the examination, &c.—since there is no reason to

discuss these questions here. All the examiners whose work is under consideration were specially trained for this work, and not employed in it until I was personally satisfied of their competence. They all used similar methods, and may, I think, be trusted to have exercised due care and diligence. The number of examinations made per case in the new series is roughly as follows:

In the Birmingham series (101 cases), all but three cases were examined thrice each. At Brighton (55 cases), most cases had only one examination, but a few two or three. At Bristol, about a half of the 227 cases studied had three examinations apiece, and the rest one or two. The Leeds and Sheffield cases (338 and 168 cases respectively) mostly received one examination each, but a number received two or more. The 120 cases at Reading were examined only once each. It is thus evident that the majority of the cases in the whole series received but one examination apiece; though a significant proportion received two or three, and a negligible number more than three.

Now from figures which I have published elsewhere (1917), from those recorded by the Liverpool workers and others, and from those which I have accumulated since, it is clear that if any series of cases is examined only once apiece, the number of infections found is very considerably less than that which actually existed. The errors due to deficient examination are not easily determinable with precision; but it is probable that, *if the series examined is sufficiently large*, they are approximately of the following order of magnitude: *A series of cases examined once apiece may be expected to disclose about one-third of the infections actually present.* The number of infections found will, at all events, almost certainly be less than one-half of the real number. The error for a series examined *thrice per case* is more easily determined: and it is probable that, in such a series, *between one-half and two-thirds of the actual number* of infections will have been detected.

From these considerations it is clear that the percentages given in the last table—since they are based, for the most part, on series examined only once per case—must be multiplied by at least two, or even by as much as three, in order to obtain the true incidence of infection. To multiply by two would almost certainly give too low a value, since a relatively small number of cases received more than one examination; but if we multiply by three, we shall run the risk of obtaining too high a figure, since this would be the factor appropriate to a series examined throughout only once apiece. It is not at present possible to deal with findings of this sort with great mathematical accuracy, but it is probably safe to assume that the true figures lie between the limits which can be approximately determined in this manner. I believe, therefore, that the following figures give a fair estimate of the true incidence of infection with the various protozoa, as indicated by the minimal actual findings exhibited in the preceding table:

Protozoon :	Estimated percentage incidence.
Entamoeba histolytica	7—10
Entamoeba coli	36—54
Endolimax nana	9—13
Iodamoeba bütschlii	0·5—0·75
Giardia intestinalis	18—27
Chilomastix mesnili	6— 9

It is now necessary to consider each intestinal protozoon in greater detail, since there are special points of interest connected with each and these have hitherto been omitted. I shall begin with *Entamoeba histolytica*, which is the most important, and which must therefore be considered in greatest detail.

1. *Entamoeba histolytica.*—It is clear that infections with this protozoon are by no means uncommon in the resident population of Britain. At least one carrier of this parasite has been found by every worker who has taken part in the present investigation. It has been demonstrated that 3·4 per cent. of examined persons who have never been abroad (over 3,000 cases) are infected ; and, as already stated, this probably indicates that some 7 to 10 per cent. of these individuals actually harboured the parasite.

If it be permissible to generalize from these findings, for a relatively small number of cases, to the population as a whole, then it appears that the people of these islands are infected to a noteworthy extent—an extent, at any rate, which was quite unsuspected until recently. It may, of course, be objected that such generalization is not yet justified. The sample examined is small : it may not be representative. On the other hand, it may be remarked that the sample, though small, is not very small ; and there is no obvious reason why it should not constitute a fair sample—unless it be that it consists mainly of persons belonging to the lower grades of society. It must not be forgotten, however, that among the higher grades it becomes increasingly difficult to find persons who have never been abroad, and who have therefore only been exposed to infection at home. When this is borne in mind, it appears not improbable that the entire population—including all who have and all who have not been abroad—would, if subjected to examination, prove to be even more heavily infected with *E. histolytica* than the figures here recorded seem to indicate. There seem no good grounds, at all events, for supposing that the incidence for the total population is less.

The first indigenous case of *E. histolytica* infection recorded in Britain is probably that of Dickinson (1862), though the diagnosis rests on indirect evidence.[1] The case of Saundby and Miller (1909) appears to be the first in which *E. histolytica* was actually seen,[2] and the case described by Wenyon (1916) was apparently the earliest recognized carrier showing cysts in the stools.[3] The later cases, described by the Liverpool workers and others, belong almost entirely to this class—more or less healthy persons (carriers) showing no clinical signs of disease due to their infections.

[1] See p. 17. [2] See p. 19. [3] See p. 7.

It is certain, from the morphology of the organism and its pathogenicity to the cat—and sometimes to human beings—that the amoebae and cysts found in British subjects really belong to the species known as *Entamoeba histolytica*—already familiar as the 'cause' of amoebic dysentery and hepatic abscess in the tropics. It has been shown (Wenyon and O'Connor (1917), Dobell and Jepps (1918), &c.) that there are different strains of this species, distinguishable by the dimensions of their cysts. No detailed study of the strains found in British infections has yet been made: but it is clear, from the measurements recorded by several workers (see Chapter III), that similar strains exist in Britain. The commonest—as in the case of the 'tropical' infections—appear to be those with cysts measuring 7–9 μ or 12–13 μ in mean diameter: but there is not yet sufficient evidence to show the relative frequency of the various strains in Britain.

Up to the present I have considered the incidence of *E. histolytica* infection in Britain *en bloc*—without distinction of age, sex, locality, or occupation of the infected individuals. Something on each of these heads must be added here.

Age.—The age at which infection with *E. histolytica* may be first acquired has not yet been established with certainty, but it is undoubtedly very early. The largest series of children examined are those of Matthews and Smith (1919), who examined 548, and of Miss Nutt, who examined 185. The first series showed that 1·8 per cent. were infected with *E. histolytica*, the second 2·7 per cent.

Matthews and Smith (1919) state that 'among fifty children, under one year of age, no infections were found': but one infection with *E. histolytica* was discovered by them 'in a girl just three years of age'. This is probably the youngest case in all the series, but Mr. Campbell has recorded (p. 31) another *E. histolytica* infection in a child of 3; and it may be recalled that Moore's patient (1881) died—apparently of an amoebic abscess of the liver—at the age of $3\frac{1}{2}$ years. It is possible that in the case recorded by Mr. Thacker (p. 28) the patient contracted amoebic dysentery when only two years old.

There is now definite evidence that infections with intestinal protozoa may be acquired during the first year of life (cf. *Giardia*, p. 52); and, although such early infection has not yet been observed in the case of *E. histolytica*, there appears to be no reason to suppose that it behaves, in this respect, differently from the other common intestinal organisms.

Infections with *E. histolytica*—and all other intestinal protozoa—appear to be remarkably persistent, and it is probable that when an infection is once acquired it lasts, in most cases, for the rest of life. (Cf. Wenyon and O'Connor (1917), Dobell and Stevenson (1918).) The longer a person lives, the longer he is exposed to the risk of becoming infected; and consequently one would expect that, *ceteris paribus*, older people would show a higher degree of infection than younger. This appears to be true. Matthews and Smith have given the following figures bearing on this point:

Age of Cases.	No. examined.	Percentage of *E. histolytica*.
1— 5	275	1·1
5—12	223	3·1
circa 18	1098	5·6

I am not sure whether these authors accept this interpretation of their figures, for they appear to believe that some intestinal infections may be commoner in children than in adults. This supposition is linked with their hypothesis that protozoal infections of the intestine do not persist indefinitely, but tend to die out in the course of time. They have not been able, however, to obtain any direct or conclusive evidence of this, and there are many observations which are not consistent with such a belief. It must be noted here, further, that the above figures are selected, and it is possible to select others which might lead to a different conclusion. The numbers constituting most of their series are comparatively small, however, and the error inherent in a system of only one examination per case is large. And consequently when results such as those recorded by Miss Nutt (p. 36) are considered—she found 2·7 per cent. of 185 children and only 1·5 per cent. of 321 adults infected with *E. histolytica*—it is by no means easy to draw certain conclusions from them. It is at least debatable whether these findings indicate—as they might seem to at first sight—that the children were more heavily infected than the adults: and on other grounds such a generalization appears to me unwarranted.

Sex.—In several series the cases have been sorted into sexes, and the findings presented for the two classes separately. The results have been consistent throughout, and show clearly that *E. histolytica* occurs in males and females with equal frequency. It will be unnecessary to recapitulate all the findings here: an example will suffice. Miss Nutt, in the Leeds series, found 1·2 per cent. of adult males infected, and the same percentage of adult females. Other series gave comparable results, and Matthews and Smith have already concluded that the distribution of *E. histolytica*—and other intestinal protozoa—is 'irrespective of sex'.

Occupation.—Matthews and Smith (1919) note that 'some of the results we have obtained seem to indicate that there may be occupational differences in the incidence of infection' with *E. histolytica*. They give the occupations of a number of the cases which they studied, and these have been recorded also for most of the individuals in the newer reports printed in the previous chapter. From all the records—including those of Baylis (1919), and others already printed elsewhere—I have been able to collect information concerning the occupation of one hundred persons found to be infected with *E. histolytica* in Britain. Not one of these had ever been abroad prior to the time of examination. I have purposely excluded from this series all children under 12, and patients in lunatic asylums. On analysing and classifying these cases I obtained the following list:

Occupation.	No.
Miners	17
Engineers, mechanics, &c.	13
Clerks, shop assistants, &c.	8
Housewives, domestic servants, &c.	6
Dock labourers, &c.	5
Farm labourers	4
Metal workers	4
Spinners and weavers	4
Carters	4
Railwaymen, porters	3
Stone-workers, &c.	3
Labourers	3
Butchers	3
Glass-workers	2
Chemical workers	2
Carpenters, &c.	2
Bootmakers	2
Packers, warehousemen	2
Schoolboys	2
Sanitary worker	1
Fisherman	1
Tailor	1
Gardener	1
Grocer	1
Greengrocer	1
Miller	1
Baker	1
No occupation (paupers)	3
Total	100

I give these figures for what they may be worth. They do not, unfortunately, give us any indication of the relative frequency of infection among different classes of workers: but they are of some interest in other ways. For example, the fact that *E. histolytica* occurs among domestic servants, bakers, butchers, grocers, and other persons who habitually handle food, suggests possibilities which cannot be ignored in considering the spread of infection through the community. (Cf. p. 54 *infra*.)

On general grounds one would expect *E. histolytica* and other intestinal protozoa to occur most frequently among people who live under the most insanitary conditions. Prompt and efficient disposal of faeces, and great personal cleanliness, would certainly limit the spread of infection. It is scarcely surprising to find that farm hands and dockers are infected, and nobody is likely to be astonished at seeing a 'sanitary worker' in the above list. It is unfortunate that no figures are available to indicate the incidence of infection among these various classes, and among groups of persons belonging to higher social strata. It will be remembered that practically all the persons hitherto studied belong to the labouring and artisan classes; and consequently all the carriers found are of the same status.

The large number of miners in the list will be noted, and this record is of particular interest. The infected individuals were mostly found by Matthews and Smith in a series of colliers. In this series, consisting of 78 men, no less than 10, or 12·8 per cent., were found to be infected with *E. histolytica*. All the infected

individuals came from the South Wales coalfields. Although the series is small this percentage is remarkably high, when it is remembered that the stools were examined only once apiece. It is probably too high to be without significance, and appears to indicate that miners are infected to a greater extent than many other classes of the community: which might, indeed, have been expected, since the localization and spread of some other parasites in mines is already well known.

The high rate of infection with *E. histolytica* found among lunatics should also be noted here. At one asylum Matthews and Smith (1919 *a*) found 9·7 per cent. of the inmates infected, as a result of making only one examination per case. This is a high rate, but not so high as a previously published record for the same asylum appeared to indicate (Yorke, 1918). This series well illustrates the possible errors in small samples. In the first report, when only 46 cases had been examined, no less than 19·5 per cent. were found infected with *E. histolytica*. Later, when 207 cases had been studied, this percentage fell to 9·7. At another asylum, where he examined 504 patients (once apiece), Smith (1919) found only 4·2 per cent. infected. Campbell (*supra*, p. 31) found 2·94 per cent. of 'mental cases', in a workhouse, infected with *E. histolytica*—as compared with 2·75 per cent. in a larger series of 'non-mental' cases in the same institution. Such figures as these do not justify the conclusion that all lunatic asylums are hotbeds of *E. histolytica* infection.

In considering the possible correlation of *E. histolytica* infection with particular occupations or modes of life, it is important to remember that the parasite occurs in children—even in those of very tender age. When this is borne in mind, and the results of the investigation of several families by Smith and Matthews [1] and Kuenen [2] are also taken into account, it seems clear that the home is no longer negligible as a source of contagion; and that occupation may, perhaps, be one of the less important of the factors which determine the acquisition of infection with *E. histolytica*.

Geographical Distribution.—In the reports already published, and in those now presented in Chapter III, there is some information about the present distribution of *E. histolytica* in Britain. It is not complete, but it is, in some respects, suggestive.

Most of the persons in whom *E. histolytica* has been found have been interrogated regarding their usual place of residence: and this has been recorded. The workers at Liverpool, Birmingham, Bristol, Brighton, Leeds, Sheffield, and Reading, have found most of their infected cases among residents in the districts in which they worked. The fact that not one of these workers failed to find local cases of infection shows that *E. histolytica* probably has a wide distribution. But by no means all the cases examined were habitually resident in the places in which their examination took place. Some of them had left their native towns or villages—to undergo military training,

[1] See p. 55. *infra*. [2] See p. 60.

and for other reasons—and were found, consequently, in places to which they did not properly belong. For example, the Welsh colliers examined by Matthews and Smith at Liverpool did not belong to this city: and it seems probable that they had contracted their infections in their native country rather than in Lancashire. Yet this is not absolutely certain, of course: and this uncertainty must be borne in mind in attempting to draw conclusions from the incomplete records which are available.

With the foregoing reservation, it may be stated that cases of indigenous *E. histolytica* infection have now been found in England—north, south, east, west, and the midlands: and in natives of Wales, Scotland, and Ireland. The records, from all sources, contain instances of the occurrence of *E. histolytica* in persons usually resident in the following counties:

England	Lancashire Yorkshire Cumberland Westmoreland Cheshire Shropshire Staffordshire Warwickshire Monmouth Gloucestershire Somerset Berkshire Hertfordshire Northamptonshire Sussex London	Wales	Carnarvon Cardigan Carmarthen Glamorgan
Scotland	East Lothian Shetland Isles	Ireland	Derry Cork

I have not thought it worth while to record the precise localities; for the incomplete records at present available do not supply sufficient evidence for attempting to estimate the geographical distribution of *E. histolytica* in detail. It is possible that the parasite is commoner in some places than in others, but there is no clear evidence of this in the records. The largest numbers of cases have been found in the places where the longest search has been made for them; and since no cases have yet been looked for in many areas, a complete survey is out of the question at the present moment.

Nevertheless, I believe that the available evidence indicates clearly that *E. histolytica* infection is prevalent throughout the length and breadth of the British Isles. Probably no region—not excepting even the smallest inhabited islands—will be found, if investigated, to be free from infection. This conclusion harmonizes with what is already known about the distribution of this parasite in the rest of the world: for the evidence is steadily accumulating to show that its occurrence is world-wide, and is not limited to any particular region of the globe or race of mankind.

Relation to Disease.—The workers at Liverpool have, apparently, found no evidence that the large number of healthy but

infected persons whom they studied were, as a class, more prone to suffer from dysentery or other intestinal diseases than uninfected persons from the same walks in life. The later workers —whose results are here recorded—have obtained concordant results. Histories of all the cases studied were obtained, as far as possible, and recorded: and inspection of these records shows that a past or present history of dysentery is apparently no commoner in persons infected with *E. histolytica* than in those who are nominally 'uninfected'. The figures are, however, for various reasons inconclusive. For example, when a person is found to have suffered in the past from 'dysentery', it is now usually impossible to ascertain its cause. The complaint was generally diagnosed on clinical grounds; and even if the patient is found later to harbour *E. histolytica* it is by no means certain that this parasite was the cause of the trouble. The patient may have suffered from bacillary dysentery, whilst remaining a 'healthy' carrier of the amoeba; or he may have acquired his infection after the attack of dysentery. A history of 'diarrhoea' is even more difficult to interpret; for there are but few who, on being closely interrogated, are found not to have suffered, upon occasion, from this common complaint. Furthermore, when a person has been examined but once, and found 'negative' for *E. histolytica*, the conclusion that he is 'not infected' is by no means warranted. Consequently, in all our series the 'negative' cases with a past history of dysentery or diarrhoea cannot furnish us with reliable statistics of the incidence of these diseases in persons uninfected with *E. histolytica*.

The findings, given for what they are worth, appear to be as follows for the infected persons so far studied. The Liverpool workers, in their first published series, state of the ten cases of *E. histolytica* infection investigated that 'none of them gave a history of dysentery'. The full histories of the cases in their later series are not recorded, but it seems unlikely that these cases were—in this respect—substantially different from those in the earlier series. Of the twenty-seven British carriers of *E. histolytica* studied at Birmingham, Brighton, Bristol, Leeds, Sheffield, and Reading, only one has a history of 'dysentery', while three are stated to have suffered from more or less serious attacks of 'diarrhoea' in the past. It is easy to pick out random samples of twenty-seven individuals from the 'uninfected' persons in the same series showing past records of diarrhoea or dysentery in approximately the same proportion. It is noteworthy, moreover, that there is not a single infected individual, in any series, with a history of hepatic abscess.[1]

(2) *Entamoeba coli*.—It is now clear that *E. coli*, the large harmless amoeba of the human bowel, occurs commonly through-

[1] I exclude the case recorded by Armitage (1919) from the 'British' series; for the patient, though he apparently contracted an amoebic abscess of the liver, followed by another in the brain, in England, and had never been in tropical or subtropical countries, was a native of New Zealand.

out the British Isles. This was to have been expected, and calls for no particular comment. It occurs in both sexes, apparently with equal frequency, and has been found in persons of all ages from the second year onwards (Matthews and Smith, Miss Nutt).

Although indigenous cases of *E. coli* infection were doubtless known to workers in this country before the war, the first recorded in the literature appear to be two described by Smith and Matthews (1917). Since then many others have been found by the same workers and others. From the records it appears probable that the true incidence of infection with this organism in the British Isles is high. Of the persons already examined, probably between one-third and one-half were infected—taking them in the aggregate. Some differences in distribution appear in the various records, but for various reasons, already considered, it is doubtful how far these are real. Campbell (p. 31) at Bristol, and Miss Nutt (p. 33) at Leeds, found the organism commoner in adults than in children: but at Sheffield Miss Nutt (p. 35) found the incidence in the two classes approximately equal. Matthews and Smith (1919, p. 364) give the following figures—based on one examination per case—for three classes of persons examined by them:

	Children.	Civilian Adults.	Army Recruits.
Percent. infected with *E. coli* ...	11·1 ...	6·7 ...	18·2

In a group of 207 asylum patients they found no less than 45·9 per cent. infected—as a result of only one examination per case. This indicates a very high rate of infection in this group. (See Matthews and Smith (1919 *a*), p. 91.) High figures were also obtained for Welsh miners and some other groups. To judge from most of the larger series, and those in which more than one examination per case was made—these series being freer from errors due to small sampling and deficient examination—it appears probable that 50 per cent. is a conservative estimate of the true incidence of infection with *E. coli* in the general population of the British Isles. At present there is no conclusive evidence indicating a localized distribution, except that derived from lunatics in asylums—to which reference has just been made.

(3) *Endolimax nana.*—This small non-pathogenic amoeba appears to be common in Britain. The first British cases of infection were found by Matthews and Smith—reported by Yorke (1918)—and by Miss D. L. Mackinnon (1918). The latter found three out of thirty-four soldiers, who 'had never been out of England', infected with this organism. Since then Matthews and Smith and all the workers under the War Office Dysentery Committee have found further infections in other classes of cases.

The percentages recorded in most series are low—the highest being 12·1 per cent. found in asylum patients by Matthews and Smith (1919 *a*). The patients were examined only once apiece:

and it may be noted that the inmates of another asylum, similarly examined by Smith (1919) subsequently, gave a percentage of only 3. Nothing else noteworthy regarding the incidence or distribution of this amoeba appears in the records.

Although the figures so far obtained indicate that only some 9 to 13 per cent. of Britons harbour this organism, there is reason to believe that this figure is too low. The organism is very small, and infections are in consequence easily overlooked; and it was not until comparatively late in the War that most protozoologists in this country became familiar with it. Careful examination of large numbers of soldiers returning from abroad has shown that *E. nana* is very common in them—being the next commonest amoeba after *E. coli*. My own records show that it probably occurs in not less than 50 per cent. of such cases.

(4) *Iodamoeba bütschlii*.—So far as records are available, this amoeba appears to be rare in Britain. It undoubtedly occurs, however, though the findings indicate that it is present in less than 1 per cent. of the population. The first indigenous infections described appear to be those found by Matthews and Smith (1919).[1] Only one case of infection has as yet been recorded in a child under twelve (Matthews and Smith), and at present the records are too meagre for it to be possible to draw any conclusions regarding the incidence of the organism in relation to sex, age, or occupation. I will merely note that this amoeba appears to be less common in British residents than in troops returning from overseas. The highest rate of infection hitherto recorded in British cases is 1 per cent.—found by Smith (1919) in a series of 504 inmates of a lunatic asylum.

The published records give figures for England and Wales only. I may add, however, that Case 393 of Baylis (1919)—as appears from his card entries, though not stated in his published paper—was infected with *I. bütschlii*, and this shows that the organism probably also occurs in Ireland. This is all that can be said at present regarding its distribution in these islands.

(5) *Dientamoeba fragilis*.—Not a single instance of infection with this small and apparently rare amoeba has been recorded in any of the series here considered. That it occurs in persons who have never left Britain is, however, certain: for I have recorded, with Miss M. W. Jepps, one case of infection in a person belonging to this category (see Jepps and Dobell, 1918). At present this is the only British case known to me.

The organism may be commoner than these findings appear to indicate, for it is small and extremely delicate, and perishes soon after leaving the body. Its cysts are still unknown, and the organism itself can only be found in soft stools examined in a very fresh condition. Consequently, even when present it is

[1] Recorded by these workers throughout under the name of 'Iodine cysts'. See my book (1919) for the history of this organism.

probably often overlooked, and negative records cannot be regarded as reliable evidence of its absence.

(6) *Giardia intestinalis.*—This flagellate—better known under the name of *Lamblia*—occurs commonly in people who have never left Britain. The first British infections were recorded by Smith and Matthews (1916), and since then it has been found by every worker who has examined cases of this class—including Miss Mackinnon (1918) and Baylis (1919), in addition to those whose work is here reported.

The chief noteworthy fact about this protozoon is that it has been found more frequently in children than in adults. The recorded figures are very striking, and may be briefly recapitulated here:

Investigator.	Place.	Percent. *G. intestinalis*	
		Adults.	Children.
Matthews and Smith[1]	Liverpool	7·0	14·1
Campbell	Bristol	3·9	16·3
Miss Nutt	Leeds	3·8	39·8
,,	Sheffield	7·2	15·8
McLean[2]	Reading	9·3	17·4

The uniformity with which *Giardia* has been found more frequently in children cannot be accidental, and it seems clear that these figures must have a real significance. But what this significance is, is not so clear. Matthews and Smith (1919) interpret their findings as indicating that infection with *Giardia* is actually commoner in children than in adults. They believe that this protozoon—and others found in the human bowel—'may disappear from the intestine in the course of time': 'it may be that the flagellate is mainly a parasite of children and becomes rarer in older people'. This may be true: but it must be remembered that failure to find a given organism in the stools is by no means the same thing as absence of it from the intestine. It is often extremely difficult to detect *Giardia* infections—in adults, at all events—by examination of the faeces: and to find an infection it may be necessary to prolong the examination until the stools have been carefully searched on dozens of occasions.[3] Figures which I have elsewhere published[4] appear to indicate that the chances are about 7 to 2 against the infection being detected by means of a single examination of the stools of the average infected individual. Further, neither Matthews and Smith, nor any other workers, have yet been able to adduce a single concrete case in which a *Giardia* infection has been proved to have died out. This organism is, in my experience, remarkably persistent. I have had one infected individual under observation for over ten years; and although it is frequently impossible to find the organism in the stools, the infection is still

[1] These figures (adults) are for 1,098 Army recruits.
[2] Percentages calculated by me from particulars entered on cards.
[3] See my previous Report (1917), where this subject is considered at length.
[4] *Ibid.* p. 18.

present, and has shown no signs of disappearing from the bowel. The other common intestinal protozoa appear to be equally persistent.

I therefore hesitate to accept this obvious explanation. I believe the figures merely show that *Giardia* is more easily found in the stools of children than in those of adults: they do not prove that children as a whole are more frequently infected. On the other hand, it appears quite probable that children, individually, when infected have heavier infections than adult individuals. This would not be surprising, and many analogous instances in which young animals show, individually, a far higher degree of protozoal infection than adults of the same species could easily be quoted. It seems not at all improbable that in children, when recently infected, *Giardia* multiplies more rapidly, and appears more copiously in the stools, than it does later in adult individuals in whom the infection has become chronic. It is perhaps easier, therefore, to find evidence of infection in children; but there is as yet no real evidence that children as a class are more frequently infected than adults, and none that *Giardia* infections tend to disappear when once established.

There is some very interesting evidence to show the age at which *Giardia* infections may be acquired. Matthews and Smith (1919) found no intestinal protozoa in infants during the first year of life: but they found several infections with *Giardia*—and also with other protozoa—in children aged between one and two years, and concluded that they 'may become infected with intestinal protozoa soon after they are twelve months old'. There is conclusive evidence now, however, that *Giardia* infections at least are contracted still earlier.

Miss Nutt, at Leeds and Sheffield, examined the stools of 25 children aged 12 months or less: and she found *Giardia* in no less than 6 of these. Her results—compiled from her cards—were as follows:

Age of Child.	No. infected with *Giardia*.
3 weeks	1
3 months	1
9 ,,	1
11 ,,	1
12 ,,	2

These findings are, I think, very remarkable. That a child only 3 weeks old may already harbour *Giardia* would hardly have been suspected. It implies gross contamination of its food with human faeces: and since all the intestinal protozoa are transmitted by similar means, it shows that—where suitably dirty conditions obtain—a child may become infected with any intestinal protozoon shortly after birth.

(7) *Chilomastix mesnili.*—This flagellate appears to be fairly common in Britain. It has been found by all the workers engaged in the present investigation, but the incidence of infection varies greatly in different records. Matthews and Smith (1919,

1919 *a*) found 19 infections in all the cases (2,137, excluding asylum patients) which they examined; a percentage of only about 0·9. The other workers, whose reports are printed here, found altogether 74 cases in 1,009 examined, or 7·3 per cent. Even when allowance is made for the fact that the first series was examined only once per case, and the second more frequently in some instances, the discrepancy in these findings is considerable. Miss Mackinnon (1918) found 1 infection in 34 'British' cases at Southampton; and Baylis (1919) records only 0·25 per cent. in his series of 400 naval cases. The highest figure recorded in any British series is 23·2 per cent., found by Matthews and Smith (1919 *a*) in 207 patients in a lunatic asylum.

The figures generally—of which the foregoing are a sample—show such variations that I am in much doubt as to their significance. I have estimated roughly (p. 42) that the total findings indicate that some 6 to 9 per cent. of the British population may harbour this flagellate: but I am not disposed to attach much importance to these figures. The only certain conclusion that emerges from the findings generally is that *Chilomastix* is apparently less common in British residents than in soldiers who have seen service abroad. In such persons I have invariably found a higher rate of infection.

The first British cases of infection with *Chilomastix* were recorded[1] by Yorke, Carter, Mackinnon, Matthews, and Smith (1917). Nothing else regarding this organism seems worthy of special comment here.

(8) *Trichomonas hominis.*—Up to the present this flagellate has been reported in one series of British cases only—the asylum patients examined by Smith (1919) at Rainhill. He found altogether six infected out of a total of 504 examined. Of the members of this series 34 had been abroad: but it appears that at least one of the patients who had never left England (Case J. Ho.) was infected with *Trichomonas*. Whether the other infected cases were 'British' or not is not stated.

In the other series examined by Smith and Matthews, and by the later workers along similar lines, there is not a single case of *Trichomonas* infection reported. None was found by any worker for the War Office Committee, and none by Baylis (1919) in his naval cases, nor by Miss Mackinnon (1918) in her series of 'British' soldiers. It thus seems clear that the flagellate occurs in persons who have never left Britain, though it is uncommon; and at present there are no figures which permit of even an approximate estimate of its frequency.

Trichomonas hominis can, as a rule, be found only in soft or liquid stools, and its cysts are at present unknown. Consequently it is not surprising that it has not been found in a large number of healthy persons with normally formed stools. Its apparent absence cannot, therefore, be taken to prove that it is as uncommon as the findings might at first sight seem to indicate.

[1] Recorded under the name *Tetramitus*.

(9) *Other Protozoa.*—No protozoa other than those just considered have yet been found in the stools of persons who have never left the British Isles. Nevertheless, I think it worth while to point out this obvious fact, as it may direct further attention to the point and possibly induce some workers to bring forward further records or undertake new investigations.

I would particularly emphasize the fact that no infections with intestinal coccidia or with *Balantidium* appear to have been reported in British residents who have never been abroad. As regards the former, it may be noted that there is one British case of supposed coccidiosis of the liver (Silcock, 1890); but this is still somewhat doubtful. I have discussed this case elsewhere, in a review of our knowledge of coccidiosis in man (1919*a*), and need say no more about it here. Since I wrote this paper, no new facts have, to my knowledge, come to light.[1]

As regards *Balantidium coli*, I would point out that it is somewhat singular that no British cases of infection with this ciliate appear to have been recorded. It is now generally accepted that *B. coli* is a parasite proper to the pig, from which animal man may occasionally acquire an accidental infection. Swineherds and others particularly associated with pigs are especially liable to balantidiosis. In parts of the British Isles—e.g. in rural Ireland—conditions conducive to infection are apparently not lacking: and one would expect human cases of balantidiosis to occur, from time to time, if the parasite were present in our native pigs. From personal experience I can say confidently that it is. I have, in fact, never examined the faeces of pigs in this country carefully without finding *Balantidium* present, often in large numbers. It seems, therefore, somewhat remarkable that no human cases of balantidiosis are yet on record—so far as I can ascertain.

The other intestinal protozoa described from man are at present too little known or too doubtful to merit special individual mention here.

B. The Spread of Infections in Britain.

Although much information concerning the incidence of protozoal infections in Britain has now been obtained, very little direct evidence is yet available to show how such infections are spread through the community. Such facts as have been elicited may be briefly mentioned here.

All the intestinal protozoa of man—with the possible exceptions of *Trichomonas hominis* and *Dientamoeba*, whose cysts are still unknown—are probably transmitted from man to man in the same manner. The active forms of all these organisms are capable of living in the intestine, where they multiply by fission. Outside the human body, however, they cannot long survive. They have

[1] An English case of intestinal 'coccidiosis' has, however, been recently described by Lockhart-Mummery and Gabriel (1919). Through the kindness of Dr. F. G. Crookshank I was able to examine sections showing the structures interpreted as coccidia, and I have no hesitation in saying that they were neither coccidia nor any other protozoa; and consequently that this case was certainly not one of coccidiosis.

but feeble powers of resistance, and are rapidly killed by drying and all conditions which differ from those under which they normally live in the bowel. If ingested in the active state by a human being they always perish. In other words, the active organisms can live in the intestine only, and are incapable of playing any direct part in the spread of infections from one host to another. But in certain circumstances—which are not yet properly understood—these active organisms can encyst in the bowel and pass out in the form of more or less resistant cysts with the faeces. It is by ingesting such cysts that a new host becomes infected.

It is thus evident that the actual source of infection is, in every case, the person who harbours active protozoa in his gut and passes their cysts in his stools: whilst the spread of infection to other persons is brought about by any or every circumstance which insures these cysts being ingested, while still fresh and living, by another individual.

It seems reasonable to assume that infections are usually acquired by swallowing food or drink which has been recently contaminated by fresh human faeces containing cysts. This contamination may conceivably occur in a variety of ways—by the agency of flies, the pollution of water with sewage, or otherwise. The present reports supply no direct evidence bearing on these problems, and it is therefore unnecessary to discuss the various possibilities here. What they do supply, however, is evidence that the contamination of food and drink with fresh human faeces—however it may occur—cannot be uncommon in this country: and it furnishes some suggestive facts which may indicate where the most important foci of infection should be sought. The evidence for the former conclusion is indirect, and is derived from the demonstration that a considerable proportion of the people of this country has actually acquired infection, in some way, with intestinal protozoa: for the latter inference, the evidence is contained in some observations of Matthews and Smith, which will be briefly considered here.

These workers (Matthews and Smith (1919), p. 365) 'in order to obtain a closer insight into the actual process of spread of these protozoal infections . . . singled out certain cases, chiefly those [children] infected with *E. histolytica*, and have examined the whole family of which each one was a member'. They state that 'in only one family' out of seven examined, were they 'able to examine the parents as well as the children and the records in this case (Family I) proved of great interest'. 'In all the other families,' they continue, 'we examined the children only. . . . It is of great importance to keep clearly in mind that these are the results of one examination only. They are therefore minimum results. At least the infections recorded were present; probably, in fact almost certainly, others also.' Their results are recorded in a table (*op. cit.*, Table V, p. 366), which need not be copied here in full. On account of its great interest, however, and as an example of the results obtained, I give below—in a modified form—the findings for Family I.

Protozoal Infections found in Family I.

Protozoa.	Parents		Children					
	J.S.	L.S.	L.S.	F.S.	Ja.S.	J.S.	M.S.	D.S.
Entamoeba histolytica	+	+	+	+	+	...	+	+
E. coli	...	+	+	+	+	+	+	...
Endolimax nana	+	+	+	+	+	...	+	+
Giardia intestinalis	...	...	...	...	...	...	+	+
Chilomastix mesnili	...	...	...	...	...	+	...	...
Ages	41	41	16	13	11	9	7	3

[+ denotes that an infection was found, ... that it was not found during the single examination made.]

Matthews and Smith conclude, from all their observations, 'that within certain families infections are much more common than in the general population of children. This is strikingly seen in Family I, where, in one family only, there are almost as many *E. histolytica* infections as we have found in the 548 cases taken from single members of different families. This applies also to the other infections, particularly to *E. coli* and *E. nana*.'

These authors have remarked further that 'there are at least two possible ways in which this state of affairs may have been brought about. (1) A single member (or some small number of members) of the family has become infected in a way at present unknown and from this source the infection has spread to other members of the family. . . . (2) The whole family, or those members of it who have similar infections, may have been infected simultaneously, for instance by all eating food from the same contaminated source.' Matthews and Smith 'think the former method the more probable': and with reference to the particular families in question they add the following information. 'With regard to the possible source of these protozoal infections it may be recorded that in each of the families investigated, one member —in six of the families the father, in the seventh a brother—had been abroad and had visited the home after going abroad. In five cases this member of the family was a soldier and in two cases a sailor. It is therefore possible that all the infections had their origin abroad. It is not at all necessary to make this supposition however, for protozoal infections, as we have shown (Matthews and Smith, 1919), are sufficiently common in all sections of the population to warrant the belief that they may have existed in this country before the war.'

Attempts were made to determine, by measurements of the cysts, how many races of *E. histolytica* and *E. coli* were present in the members of two families. From the few observations which they were able to make Matthews and Smith concluded that 'the evidence does not go far, but such as it is, it points to one race, and therefore presumably one source of infection, for the *E. histolytica* infection in the two members examined of Family II, and to two sources of infection in the four members of Family I'.

There is but little that can profitably be added to the foregoing observations at the present moment. In this connexion, however,

I would call attention to the similar observations recently made in Holland by Kuenen (1918)—considered more fully on p. 60; and I would emphasize once more two of the important facts which the present reports have revealed. These are, first, that intestinal protozoa are far from uncommon in British children in whom they occur even in the first year of life. The statement of Matthews and Smith (1919, p. 364 n.) that 'liability to infection does not appear to occur until the child has entered his second year' can no longer be regarded as a true proposition (see p. 52); and the possibility of infection at a very early age will in future always have to be considered in any discussion of the means by which intestinal protozoa are disseminated. Secondly, we now know that females are not less subject to infection than males; and that domestic servants, cooks, and other female members of the household may harbour *E. histolytica* and other protozoa.

We have seen also (p. 45) that the purveyors of food for domestic consumption—such as butchers and bakers—are also sometimes infected with *E. histolytica* and other intestinal protozoa, and they can certainly acquire such organisms without going abroad. There is also some evidence that people who are closely associated for considerable periods, and under conditions which are not sanitarily perfect, are apparently apt to acquire infections from one another. At all events, the high rates of infection found among colliers (see p. 45) and the inmates of lunatic asylums (p. 46) appear to point in this direction.

It appears more than probable that all the common intestinal protozoa of man occur indigenously in Britain; and with obvious sources of contagion close at hand it thus appears no longer necessary to seek a foreign origin for every case of protozoal infection found in this country.

CHAPTER V. RECENT OBSERVATIONS ON THE OCCURRENCE OF *E. HISTOLYTICA* IN FRANCE, HOLLAND, AND GERMANY.

A DISCUSSION of the occurrence of *E. histolytica* and the other intestinal protozoa of man in Europe generally, does not properly come within the scope of the present Report. But since Britain is a part of Europe, and since the British findings are of more than local interest, it seems to me desirable to consider, before I end, some comparable findings which have been recorded by our nearest European neighbours. I shall therefore give here, as briefly as possible, the recent results obtained, along similar lines, by workers in France, Holland, and Germany—these being the only adjacent countries from which, so far as I am aware, any information is yet available.

No comprehensive survey of the intestinal protozoa harboured by the native population appears to have been yet attempted in any European country save Britain; and it has seemed to me

unnecessary to collect, at the present moment, all the scraps of information previously published which appear to indicate that most of the common intestinal protozoa of man are indigenous in Europe generally. But the large number of native British infections with *E. histolytica* revealed in the present Reports must be considered in relation to the incidence of infection with this parasite in the world at large—else we may form a very erroneous opinion of its significance. The other intestinal protozoa found in Britain should also, of course, be considered in a similar manner: but as *E. histolytica* is, for practical reasons, more important, and as information about the others is even more scanty, I shall here leave them out of account.

The notes which follow are merely notes, and make no pretensions to being an exhaustive survey of the literature.

The Occurrence of E. histolytica *in France.*—There is now evidence to prove that *E. histolytica* occurs indigenously in the population of France. The evidence is chiefly derived from records of sporadic cases of amoebic dysentery and liver abscess in persons who have never left France.

Galliard and Brumpt (1912) have published the results of a careful study of an indigenous case of amoebic dysentery. Their patient was a young male Parisian, aged 25, suffering from acute dysentery and passing numerous amoebae in his stools. The observations and experiments of these workers show conclusively that the amoeba found was *E. histolytica*. In a brief survey of the literature, the authors were able to find five earlier recorded cases which, in their opinion, are to be interpreted as indigenous French cases of disease due to *E. histolytica* (by these authors called '*Amoeba dysenteriae*').

In the following year Paviot and Garin (1913) reported another indigenous case of amoebic dysentery observed at Lyons. The amoebae found in their patient—who died of the disease—appear to have been undoubtedly *E. histolytica*. (I assume that some of the authors' observations were inexact.) These authors also refer to a number of apparently similar previous cases.

A little later Landouzy and Debré (1914) described an indigenous fatal case of amoebic abscess of the liver, following dysentery. The patient was a bargee who had never left France. The clinical and *post-mortem* findings leave no doubt that this also was a case of *E. histolytica* infection. In a useful table the authors summarize previously published observations on 13 apparently similar indigenous cases of *E. histolytica* infection recorded in France. They also add a warning of the danger which may threaten the community as a result of the importation of large bodies of colonial troops, infected with the parasite, into France during the War.

Some interesting observations were recorded two years later by Ravaut and Krolunitski (1916). They stated—but without giving details—that since the outbreak of the War they had found 25 cases of *E. histolytica* infection 'of indisputably indi-

genous origin'. (They call the parasites, however, '*Amoeba Histolytica*' or 'A. H.') This large number of French cases was discovered within the limits of only a 'small area' and 'in the space of several weeks'. These authors carried out an investigation of the stools of certain 'individuals who did not show, at the time of examination, any dysenteric symptoms'. They examined, in all, 200 such persons, with the following results:

Of 72 orderlies in Hospital S. (which had received many cases of dysentery), 10 were found to be passing cysts of *E. histolytica*. Out of these 10 cases, only 4 had a previous history of having suffered from intestinal ailments.

Of 32 orderlies in Hospital G. (in which dysentery cases had been 'relatively very rare'), 2 showed cysts of *E. histolytica* in their stools. Only 1 of these had a previous history of dysentery.

Of 96 soldiers, who were in these two hospitals for various ailments, 22 showed *E. histolytica* cysts. A dysenteric history was elicited for 19 of these cases.

Among these 200 persons there were thus, in all, 34 individuals infected with *E. histolytica*. Of these, 24 had a dysenteric history, while 10 had apparently shown no symptoms due to their infections—i.e. were 'healthy' contact carriers. From these findings Ravaut and Krolunitski drew the conclusion that as many as 5 per cent. of healthy persons, in the region studied, are infected with *E. histolytica*. Although this conclusion has been copied—and apparently accepted—by some reviewers of these results, it is evident that the findings recorded do not warrant such a deduction. They are of considerable interest; but obviously they do not indicate what percentage of healthy persons, with no history of dysentery, is infected with *E. histolytica*: and the authors' comparison of these findings with the results of Mathis—who found 8 per cent. of such persons infected in Tonkin—is hardly possible. It should be added that the histories of the individuals in these series are not recorded, so that it is not possible to state how many—if any—of them can be regarded as indigenous cases of *E. histolytica* infection.

Labbé (1919) has recently recorded 8 cases of 'amoebic dysentery' which he has observed in Paris. (The author refers to the finding of 'amoebae' and 'cysts' in the stools, but does not mention the species to which they belonged.) He states that 'sometimes the disease might have been contracted during a sojourn outside of France', but 'sometimes the amoebiasis was contracted in France—even in Paris'. Though his cases may be cases of amoebic dysentery which occurred in France, the particulars recorded do not show how many of the patients studied had really acquired their infections in that country. (Only one case is definitely stated never to have been abroad, and at least two appear to have contracted their dysentery in the tropics.)

The Occurrence of E. histolytica *in Holland.*—About two years

ago some interesting observations were made and recorded in Holland by Kuenen (1918). As practically no notice has hitherto been taken of his work, I propose to summarize it here.

Kuenen, who appears to have been unaware of the observations made in this country, is already known for his careful work on amoebiasis in the Dutch East Indies. Having there studied the intestinal protozoa of Europeans and natives, it occurred to him, on his return to Holland, that similar investigations carried out at home might yield interesting results. He therefore began to study the stools of certain hospital patients in Amsterdam. Before long he found some who were passing cysts of *E. histolytica*, and although some of these carriers were found—as was to be expected—to be persons who had been in the tropics and there suffered from dysentery, he also, to his surprise, found several cases of *E. histolytica* infection in Dutch residents who had never been abroad. The following is a short summary of the indigenous cases recorded.

Case 1.—A dock-worker. Though *E. histolytica* cysts were repeatedly found in his stools, he showed no symptoms referable to his infection during his stay in hospital. He had never been abroad, but had worked on board ships plying between Holland and the Indies, and had therefore very probably been in contact with colonial carriers of the parasite.

Case 2.—A military patient—a miner by calling. No intestinal symptoms could be elicited, and no history of association with friends or relatives who had been in the Dutch Indies. *E. histolytica* cysts were present in the stools.

Case 3.—A boy of 12, from Amsterdam. He had been suffering from dysentery, with intermissions and relapses, for 8 months. Typical *E. histolytica* amoebae were found in the bloody mucous stools.

Case 4.—Another boy of 12, also from Amsterdam. Had suffered from diarrhoea or dysentery, with intermissions, for 5 months. At different times he passed typical adult, precystic, and encysted forms of *E. histolytica* in his stools.

The last two patients (Cases 3 and 4) had scarcely ever spent a single day out of their native city. They were unrelated and unacquainted with one another. The stools of all the other members of their families were examined, but none of them found infected with *E. histolytica*. The father of Case 3 had spent 3 weeks in the Indies some 20 years previously; but apart from this no indications of a possible colonial contamination through other members of the two families could be discovered. Both patients were treated with emetine hydrochloride hypodermically, and were cured of their symptoms but remained carriers of the parasite.

Case 5.—A married Amsterdam woman of 32. Since 18 years old she had had attacks of diarrhoea, sometimes with blood and mucus. Many cysts and precystic forms of *E. histolytica* were found in her loose stools. No contact with relatives from the Indies could be established. The husband and two daughters of

this patient were also examined. The husband was apparently free: the children were, however, both infected (Cases 6 and 7).

Case 6.—Daughter of preceding. She had occasionally suffered from bowel trouble, and had passed some blood and slime in the previous year. Typical *E. histolytica* cysts were present in the stools.

Case 7.—Daughter of Case 5. No symptoms of intestinal trouble. A few *E. histolytica* cysts found in the stools.

Case 8.—A married woman, aged 32, a native of Wormerveer, now living at Haarlem. Since the age of 18 she had suffered from occasional severe attacks of diarrhœa. Large numbers of *E. histolytica* cysts were found in her stools. The husband and the son of this patient were also examined, but not found to be infected.

Kuenen discusses these cases at some length; but I will merely note here that he concludes—in my opinion very properly—that it is a mistake to regard *E. histolytica* as a parasite peculiar to the tropics, and to seek an imported origin for all infections discovered in Holland. *E. histolytica* probably occurs indigenously in that country; and if, as Kuenen believes, Case 5 contaminated her two children (Cases 6 and 7), infection can evidently be spread there without foreign intervention.

It may be added that Nolen (1918) was reminded, by Kuenen's paper, of a case which he studied in 1908. His patient was a Dutch fisherman, aged 42, belonging to Vlaardingen, who had suffered for 2 years from dysentery. In his bloody mucous stools numerous active amoebae containing red blood corpuscles were found. This was doubtless, therefore, a case of true amoebic dysentery. Its interest lies in the circumstance that the patient had never been, in the exercise of his calling, further afield than England. He had never been in the tropics or in Eastern Europe, and must therefore have contracted his infection either in Holland or in England.

The Occurrence of E. histolytica *in Germany.*—In a recent paper Fischer (1920) has recorded several cases of indigenous amoebic dysentery in Germany. This observer—who has had experience of amoebic dysentery in China—appears to be unaware of the work already accomplished in England, France, and Holland: and it is clear from his paper that he is not abreast of the present state of knowledge of the amoebae of man. His findings, in so far as they are of interest in this connexion, are briefly as follows:

The author has examined the stools of 120 patients, suffering from various complaints (mostly stomach and bowel affections) in a hospital at Göttingen. He found *E. histolytica* in 2 (or 3?) cases, and other intestinal protozoa in several others. The positive cases were the following:

Case 1.—A young woman of 18, with clinical dysentery. In the bloody mucous stools amoebae containing red blood corpuscles were found, but no cysts. They were diagnosed as *E. histolytica*.

It is stated that this is an 'autochthonous' case of amoebic dysentery, but details of previous history are not given; it being merely noted that 'contact with amoebic dysentery patients could not be established'. The administration of emetine appears to have had no curative effect.

Case 2.—A 'young' man (no age stated) who had suffered from clinical dysentery in the army. He had had frequent stools—sometimes containing mucus, but no blood—accompanied by tenesmus, for a year and a half. Amoebae (none containing red blood corpuscles) and cysts measuring 10–12 μ in diameter were found in the stools, and identified as those of *E. histolytica*. No other essential facts are recorded.

Case 3.—Regarded by the author as doubtful. A man, aged 24, suffering from 'colitis'. He had served in the army from 1914 to 1918. In his stools 'a few amoebae with phagocytized red blood corpuscles' were found—also, it may be noted, macrophages containing the same inclusions. No cysts were found, and no other essential details are given.

Although Fischer calls all these cases indigenous, and says they prove that there are 'actually autochthonous cases of amoebic dysentery in Germany', no proof of these statements is supplied in the present paper. The author merely records cases of amoebic dysentery, or amoebic infection, detected in Germany. Further evidence is necessary to establish the fact that the infections were contracted in that country. The author does not allude to any earlier observations bearing upon this subject, but I may mention that at least one indigenous case appears to have been previously recorded. For Jürgens (1906) states that he performed a *post-mortem* examination on a boy of 16, at the Charité Hospital (Berlin), who had lived in Germany all his life but presented, notwithstanding, typical lesions of amoebic dysentery.

The foregoing observations, while they show the necessity of further work along similar lines, make it clear, I believe, that *E. histolytica* occurs indigenously in France, Holland, and Germany. They supply no exact figures for comparison with those now available for Britain, but they indicate clearly that the conditions found in this country are not peculiar. Indeed, they make it highly probable that the incidence of infection with *E. histolytica*—and other intestinal protozoa—within our shores is not greater, nor the occurrence of amoebic diseases commoner, than in the countries inhabited by our nearest neighbours in Europe.

CHAPTER VI. CONCLUDING REMARKS.

The observations recorded in foregoing chapters have demonstrated conclusively that all the commoner intestinal protozoa of man occur now in the population of the British Isles. The species actually recorded, up to the present moment, are the following:

Rhizopoda (Amoebaea)	1. *Entamoeba histolytica.*
	2. *Entamoeba coli.*
	3. *Endolimax nana.*
	4. *Iodamoeba butschlii.*
	5. *Dientamoeba fragilis.*
Mastigophora (Flagellata)	6. *Giardia intestinalis.*
	7. *Chilomastix mesnili.*
	8. *Trichomonas hominis.*

Infections with organisms belonging to the other main subdivisions of the Protozoa—the Sporozoa (e.g. Coccidia) and Ciliophora (e.g. *Balantidium*)—have not yet been recorded.

The probable frequency with which the above protozoa occur in Britain has been discussed in Chapter IV, and need not be considered again here. I would only remark that the estimates at which I have arrived are based upon the results of an examination of the stools of over 3,000 British residents, of all ages and both sexes, who had never left these islands. They therefore have a substantial basis.

From a practical standpoint, the most important figures are those relating to the incidence of infection with *Entamoeba histolytica*—the 'dysentery amoeba'. It has been demonstrated that not less than 3·4 per cent. of the persons already examined harbour this parasite; and it is probable that this figure—which represents an absolute minimum—indicates that the true rate of infection is of the order of 7 to 10 per cent.

The evidence now obtained appears to show clearly that *E. histolytica*—as well as many other intestinal protozoa—occurs indigenously in Britain. It has been suggested, however, that the occurrence of large numbers of carriers of this parasite among our home population at the present day is a consequence of the War. There is, indeed, good evidence to prove that our troops returning from the tropics, and other places where *E. histolytica* is known to be endemic, were often heavily infected; and it is true that an extensive infection of the resident civil population of Britain has been demonstrated only since the outbreak of the War. Consequently, the present state of affairs may be due to the contamination of the civil population by an infected soldiery returning home. This conclusion is, I believe, unjustifiable and almost certainly incorrect. All the evidence is against it. We know—from the early records considered in Chapter II—that long before the War *E. histolytica* occurred in British residents

who had never been abroad. We know that the parasite occurs in widely separated parts of the country, and is comparatively common. We know that it occurs at the present time in every part of the world where it has been sought; and from its relative harmlessness to human beings it seems probable that its association with mankind is no new thing. The very fact that *E. histolytica* is now comparatively common in persons who have never left the country, whilst amoebic diseases are uncommon, argues a degree of tolerance in the community such as is inconsistent with the supposition of its recent importation. Finally, it has not been possible, in those cases where careful inquiries have been made, to establish any direct connexion between the native cases of infection and carriers returning from abroad.

The proof that *E. histolytica* and other intestinal protozoa occur not uncommonly in the resident civil population of these islands offers a wide and somewhat novel prospect for contemplation by the British physician. The organisms themselves are—save to those who have studied tropical medicine—comparatively unfamiliar, and consequently apt to be regarded with suspicion. Medical practitioners in this country will therefore desire some further information concerning the protozoa in question, and will naturally ask what all the figures in this Report really mean—whether they have any practical importance, and, if so, what it is. To answer such questions finally is hardly possible at the present moment. Nevertheless, approximate answers can, I think, even now be found; and in the remainder of this chapter I propose to give my own general conclusions from the findings before us, and to discuss very briefly certain problems and points of interest which they suggest.

In the first place, the figures mean—and to my mind this is their worst meaning—that the inhabitants of Britain must frequently consume food and drink which has been exposed to gross contamination with fresh human faeces. The findings demonstrate this conclusively. Of the protozoa here considered, not one lives—so far as we know at present—anywhere but in the human bowel. The cysts of all are discharged in human faeces only; they cannot long survive outside the human body; and they cannot withstand drying. Infection can only be acquired, in natural conditions, by swallowing fresh, undried cysts derived from the faeces of a human being. Consequently, when we find persons infected with intestinal protozoa we must conclude that they have previously swallowed particles of the fairly fresh and damp excrement of other persons; and when we find many people presenting such evidence of pollution, we can only conclude that the disposal of human faeces has not yet been brought generally to that pitch of perfection which seems desirable.

The exact mechanism by which *E. histolytica* and other intestinal protozoa are spread through the community is not yet fully understood; but from the facts now available it is clear that in the past we have been too prone to lay stress upon foreign sources of infection. We have ignored the fact that we ourselves harbour,

and have probably for centuries harboured, an abundance of protozoa. If, at the present moment, a case of amoebic dysentery were discovered in a British household, it is probable that an attempt would be made to trace the origin of the infection to some male member of the family who happened to have been abroad—especially if he had served in the Army and there suffered from 'dysentery'. But it must, in future, be realized that the source of infection is not necessarily the soldier son, returned from foreign parts. It may equally well be the mother of the family, who has never been abroad; it may be the nursemaid or the cook—with no signs of dysentery about them: it may be the greengrocer who handles the uncooked vegetables and fruit consumed by the family. All these hitherto unsuspected persons may be passing cysts of *E. histolytica* and other protozoa by thousands every day. Certain vocations, sojourn abroad, and some other obvious factors are no doubt rightly regarded with suspicion when considering possible sources of infection: but in future suspicion will also fall upon the home circle, and the mere circumstances that a man lives at home, does not work in the sewers, has not had dysentery, and has never been abroad, can hardly be regarded as sound reasons for believing that he is, therefore, *not* infected with *E. histolytica*.

The foregoing points are chiefly for the consideration of the sanitarian, but there is another which is worthy of notice by the statistician also. This is that the figures now obtained offer us a means of checking and correcting certain others which have been previously arrived at. If a body of troops returning home is subjected to protozoological examination, the results obtained can now be more accurately interpreted than has been possible hitherto. For example, if, in such troops, it is found that some 10 per cent. are infected with *E. histolytica*, it is no longer easy to argue that they have become contaminated abroad; or that they are importing foreign parasites and thereby threatening danger to the rest of the community. Such a figure would, indeed, be almost devoid of significance, since we now have evidence that the men concerned might have been already infected to a similar extent before ever they set out from home. The earlier records of the incidence of protozoal infections in our returning troops will all have to be reinterpreted in the light of the new observations.

The clinician will probably be more concerned with knowing whether intestinal protozoa cause diseases, and if so, whether these are sufficiently serious to be worthy of his attention. Fortunately, our present knowledge, though imperfect, is able to supply fairly satisfactory answers to these questions. It has now been proved that two of the intestinal protozoa of man are facultatively pathogenic—namely, *Entamoeba histolytica* and *Balantidium coli*. The latter has not yet been found in man in Britain (i.e. as an indigenous infection): the former has, however, been shown to be fairly common in these islands; and as it can—in certain circumstances—cause dysentery, liver abscess, and other diseases,

it obviously merits very careful attention. There is as yet no conclusive evidence to prove that any of the other intestinal protozoa of man are pathogenic; while there is much evidence, on the other hand, to show that they are, in the majority of cases, harmless. As possible causes of human disease they may, therefore, all be left out of account for the present.

As regards *E. histolytica*, the evidence clearly shows that this parasite is not responsible for any considerable proportion of the disease of this country, though it shows equally clearly that it is responsible for a small number of cases. This statement is, however, in all probability applicable to all other countries where the parasite is known to occur. Britain is not in a peculiar position in this respect.

The fact that the majority of indigenous infections with *E. histolytica* have been found in comparatively healthy individuals, with no past or present symptoms of dysentery or liver abscess, has led some people to wonder whether the '*E. histolytica*' of natives of Great Britain is, after all, the real *E. histolytica* of the tropics—the amoeba which causes amoebic dysentery and other diseases. There is, however, no longer any room for doubt on this subject. It can be confidently asserted that the British parasite *is* of the same species as the amoeba of tropical amoebic dysentery. It is morphologically indistinguishable; and it is similarly a facultatively pathogenic parasite—as the indigenous human cases of amoebic dysentery and liver abscess indicate, and as its transmission from healthy human carriers to the cat confirms. In this animal the organism causes acute amoebic dysentery indistinguishable from that produced by tropical strains of the parasite.

Although there can be no doubt as to the identity of the British parasite, there is possibly still room for wonder at the fact that, although some 7 to 10 per cent. of the population is infected, yet amoebic dysentery and liver abscess are rarities in this country. But in contemplating this apparent discrepancy the following points have been all too often overlooked. First, infection with *E. histolytica* does not invariably, or even usually, result in dysentery—even in the tropics or other places where 'amoebiasis' is reputed to be 'endemic'. The belief that it does was based upon faulty knowledge of the life-history of the parasite and its relations to its host,[1] and it is now certain that it is the exception, and not the rule, for *E. histolytica* to cause dysentery or liver abscess in human beings. (This does not mean, of course, that it does not usually cause ulceration or erosion of its host's intestine. It must always do this, as it is an obligatory tissue-parasite: but the injury of the intestine is not usually sufficient to give rise to any clinically recognizable symptoms.) The conditions observed in England are thus not really peculiar. They merely illustrate a special instance of a general phenomenon.

[1] I need not labour the point here. The facts now are, or should be, well known. The reader who seeks further information on the subject will find it in my book on the amoebae of man (1919).

In the second place, it must be remembered that it has not yet been proved that amoebic diseases are so excessively rare in Britain as they are usually assumed to be. We know from the observations of Dickinson, Moore, Saundby and Miller, Marshall, and Worster-Drought and Rosewarne, that both amoebic dysentery and liver abscess occur in persons who have never left this country.[1] We can hardly doubt, from the more recent observations of Laidlaw (1918), that British cases of amoebic disease will be found to be more frequent when they are more frequently and carefully looked for. The work of Bartlett (1917), Laidlaw (1918), and others, suggests that some at least of the cases hitherto diagnosed as 'ulcerative colitis' by British physicians may be really caused by *E. histolytica*. It is, indeed, almost impossible to read such works as those of Gemmel (1898) or Hawkins (1909) without suspecting that some of their patients suffered, though they did not realize it, from amoebic dysentery. What other conclusion can we draw, for example, from Gemmel's 80 autopsies on cases of 'idiopathic ulcerative colitis'—containing two dysenteric patients displaying ulcerated large intestines coupled with abscesses in their livers? Furthermore, intestinal disturbances, such as constipation and diarrhoea, are the commonest symptoms of infection with *E. histolytica*; and few practitioners will be prepared to prove that among their patients suffering from these everyday complaints there are none whose symptoms are due to amoebic infection. Again, from the recent observations of Smith (1919) it appears probable that some of the 'asylum dysentery' in England may be amoebic[2]—though from the work of Gettings and others it seems probable that the proportion is inconsiderable.

It thus seems reasonable to conclude that amoebic diseases are rare in Britain, as they probably are everywhere else in the world—rare, that is to say, in proportion to the number of persons infected with the parasite which 'causes' them—yet not so extremely rare as defective observations and inadequate statistics might lead one, at first sight, to suppose.

Thirdly, there is another point which should not be overlooked. Although it now appears certain that 'pathogenic' and 'non-pathogenic' strains of *E. histolytica* do not exist[3]—all strains of the species being tissue-parasites and consequently capable of being 'pathogenic' to a susceptible host—nevertheless it does not follow that all strains are equally 'harmless' to all hosts. Amoebic dysentery is the consequence of an incompatibility between the amoebae and their host: it is an abnormal or 'diseased' condition for both. The carrier of the parasite is a compatible host—one adapted to life with the amoebae, and one to whom the amoebae themselves are adapted. Now it appears probable that *E. histolytica* has parasitized man for a very long time. This is indicated by its geographical distribu-

[1] See Chapter II.

[2] The observations of Gemmel (1898)—alluded to above—also seem to point to the same conclusion.

[3] Cf. Dobell (1919), p. 58 *et seq.*

tion—apparently world-wide; by its frequency—a considerable percentage of every race of man properly studied having been found infected; and by the fact that its normal host is the 'healthy' carrier. In the course of ages *E. histolytica* has become well adapted to man, and man well adapted to *E. histolytica*. But there are many races of Man, and there are probably many races of *E. histolytica*—some of these, indeed, being already easily recognizable by the dimensions of their cysts. On analogy with other protozoa it is probable that there are also many physiologically different races—at present indistinguishable. It is therefore by no means an extravagant hypothesis to suppose that, in the course of time, particular races of amoebae have become specially adapted to particular races of human beings. The races of *E. histolytica* occurring in Britain may well be peculiarly suited to a life in Britons: the races in Africa to African natives. But if the Briton goes to Africa, or if the African comes to Britain, and acquires the parasite indigenous to the country, it is possible that these new combinations might produce a different result. It is possible that African strains of amoebae may be unsuited to a life in British bowels, and may in this abnormal environment give rise to dysentery more readily than they do in their native habitation in the negro. I will not assert that this is the correct or complete explanation of what appear at present to be the facts regarding the incidence of amoebic diseases; but I think that some such hypothesis as the foregoing offers the most plausible line of interpretation, and I know of no other which is consistent with all the established facts—many of which are very curious and, at first sight, contradictory.

I would emphasize here once more that the observations already made in Britain, in France, in Holland, and in adjacent European countries, all tend to show most clearly that *E. histolytica* is as much at home in Western Europe as it is in the tropics. The distribution of this amoeba is almost certainly world-wide. It probably occurs now wherever there are men, and has so occurred from time immemorial. *E. histolytica*, like the eagle of Prometheus, preys upon the perpetually regenerated flesh of the human body; and in the words quoted by 'the beloved Physician', 'Wheresoever the body is, thither will the eagles be gathered together.'

In conclusion, I will sum up in a few words the present position with regard to *E. histolytica*—as I conceive it—in so far as it concerns the population and practitioners of this country.

Entamoeba histolytica is, or may be, a 'cause' of human disease; but usually it is comparatively harmless to the persons it inhabits—at all events, in a population in which it has been long established. The parasite occurs commonly in Britain, in the resident native population, but to the majority of its hosts it causes little or no inconvenience. It is probably not a new or recently imported parasite, but at least as old an inhabitant of these islands as the British nation. It has apparently caused but little trouble in the past, and is not likely to cause more in the

future. Its prevalence, therefore, need not be regarded with alarm. Nevertheless, the frequency with which it still occurs indicates that conditions are still suitable for its dispersal: and as these conditions imply personal uncleanliness or faulty sanitation, it is clear that, as a nation, we still have much to learn about individual and communal hygiene.

Moreover, though *E. histolytica* is often comparatively innocuous, it is harmful and even dangerous to certain abnormally susceptible individuals; and if such persons are rare, they are, nevertheless, not negligible. Consequently, it will be necessary in future for British physicians to remember *E histolytica* when they are called upon to treat cases of dysentery, chronic diarrhoea, 'ulcerative colitis', or other intestinal ailments which may possibly be due to this parasite; and to remember it also when they encounter patients displaying symptoms of hepatitis, hepatic abscess, and similar disorders, in the causation of which *E. histolytica* plays a part. Amoebic dysentery is a disease which, if promptly and properly treated, is usually amenable to specific therapy: but one which, when neglected or mistreated, often becomes chronic, leads to much misery and unnecessary suffering, and frequently terminates fatally.

Fortunately, modern methods of diagnosis have made it possible to recognize the amoebic diseases with certainty: and if they have also revealed possibilities of trouble which have lain hitherto unsuspected, they have at the same time put our knowledge of both the cause and the cure of these diseases on a sound scientific foundation. Therefore, let the British physician grieve not, nor be filled with forebodings at the outcome of the work chronicled in these pages: but let him rather rejoice that it gives him new light and new weapons—if he will but use them—for his fight against disease and death.

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[1] This name is misprinted as 'Taylor' in the original.

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Milk and its Hygienic Relations, by Janet E. Lane-Claypon, M.D., D.Sc. (Lond.). Price 9*s.* net. [Longmans, Green & Co.]

The Amoebae living in Man, by Clifford Dobell, F.R.S. Price 7*s.* 6*d.* net. [Bale, Sons & Danielsson, Ltd.]

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